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A PROSPECTIVE STUDY TO ASSESS THE FUNCTIONAL OUTCOME FOLLOWING ORIF VS PERCUTANEOUS FIXATION IN SCAPHOID FRACTURE

Orthopaedics

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ABSTRACT

The study aimed to assess clinical, radiological as well as functional outcome in patients of scaphoid fracture following open reduction as compared to percutaneous fixation with Herbert Screw. This study was conducted at Department of orthopedics, for 1 year on 30 patients of scaphoid fracture. Fractures were first tried for percutaneous fixation using volar approach, ORIF with volar approach was used when adequate reduction was not achieved. Patients were followed at 2 week interval till union. Clinical assessment at final followup was performed using MMWS. The mean mean duration of presentation after injury of 19.9 days (3 to 162 days). The mean MMWS score was 93.8 (90-100) for percutaneous and 83.8 (70-95) for ORIF. Since fracture treated with percutaneous fixation are associated with early union and early return to functional activity as compared to ORIF, Herbert screw for fixation with percutaneous technique for scaphoid fracture must be encouraged for displaced or undisplaced fracture.

KEYWORDS

Percutaneous Fixation, Orif, Mmws, Scaphoid Fracture, Bhopal

INTRODUCTION:

The word scaphoid has been derived from a Greek word *"skaphos"* which mean boat due to its shape.[1] The scaphoid bone fracture account to about 80 to 90% fracture of all carpal bones. Scaphoid fracture are commonly encountered in young, adult males.[2] The management of displaced, comminuted, and unstable fractures is only surgical intervention, however, management of undisplaced or minimally displaced surgical fracture is controversial. Literature suggests that patients with undisplaced fracture of scaphoid must be initially managed with the help of cast immobilization for 2 to 3 months.[3,4,5] Casting is an ancient technique that has traditionally been reserved for distal pole fractures and nondisplaced waist fractures, with union reported to occur at a mean of 53 days and 65 days respectively.[6] Cast has been associated with longer time of union which inturn has multiple drawbacks which include sclerosis, comminution, translation and location in the proximal pole.[6] Apart from non union, other complications of displaced fracture of scaphoid include increased rate of redisplacement and delayed union, when managed with cast immobilization alone.[7,8]

Alternative management of scaphoid fracture include early operative intervention with screw fixation which not only limits the need for a cast, but may also allow earlier return to sports and work.[5,9,10] The rate of complications such as non union are much lower for non displaced fractures and complication rate are almost nil when a non displaced fracture is adequately treated and protected.[11] However non union rate for displaced fractures is approximately 50% as compared to 10% non union rate of undisplaced fracture.[3,12,13] The occurrence of non union is responsible for various sequlae i.e. altered carpal biomechanics, pain, diminished range of motion of wrist, poor grip strength etc.[14] Since rates of nonunion scaphoid fractures treated with conservative management are quite high, thus, open reduction with internal fixation (ORIF) has been recommended.[15,16]

Open reduction and internal fixation technique for management of fracture of scaphoid was first introduced by McLaughlin[17] and then the results were reproduced positively by various other studies.[18] Percutaneous fixation is also an alternative and simple technique for management of scaphoid fracture.[19, 20] Both the methods have shown superiority over management of displaced fracture when compared to cast immobilization. The present study aimed to assess clinical, radiological as well as functional outcome in patients of scaphoid fracture following open reduction as compared to percutaneous fixation with Herbert Screw.

METHODS:

The present study was conducted as an interventional study at a tertiary care Centre, Bhopal for a period of 2 years i.e. from 1st November 2017 to 20th October 2019. A total of 34 cases of scaphoid fracture reported to the study area during the study period. Inclusion criteria was patients of acute scaphoid fracture, patients with delayed union or fractures showing no sign of healing after 12 weeks of cast immobilization. Patients with distal radial tuberosity fracture, or presenting with osteonecrosis, patients with previous wrist injury or any other associated fracture around the wrist were excluded.

Written consent was obtained from all the study participants. The present study was conducted on 30 patients out of 34 cases of scaphoid fracture fulfilling the inclusion criteria. All the cases were treated using Herbert screw. Patients were subjected to X ray wrist postero-anterior view, lateral view, semipronation oblique and antero-posterior view to assess the scaphoid fracture. Injuries were graded according to Herbert and Fisher Classification.

All fractures were first tried for percutaneous fixation using volar approach, patients in whom adequate reduction was not achieved with percutaneous method, ORIF with volar approach was used. However patients with history of fracture more than 5 months were managed with ORIF and bone grafting directly.

Post operatively, cast immobilization was done for all the patients. Patients were followed at 2 weeks, 6 weeks and 10 weeks and then every two weeks till fracture union post operatively. The minimum follow up was twelve months. After 2 weeks of operative management, sutures were removed whereas cast was removed at 6 weeks and a removable wrist immobilizer brace was tied for next four weeks. Also all patients were advised physiotherapy with hand grip strengthening exercise and active assisted range of motion exercise.

At each follow up, clinical and radiological examination was conducted to assess the healing and union. Union was considered to have occurred when there was no tenderness at the anatomical snuff box or at scaphoid tubercle and there was evidence of trabeculae crossing fracture on at least two views. Clinical assessment at final follow up was performed using Modified Mayo Wrist Score (MMWS). To assess grip strength, patients were asked to squeeze the examiners index finger, and the strength was compared on contralateral side. Range of motion was measured using goniometer.

Data analysis- Data was compiled using Ms Excel and analysed using IBM SPSS software version 20. Numerical variables were expressed as mean and standard deviation. MMWS score was calculated for each patient for ORIF and percutaneous method separately.

RESULTS:

The present study included 30 patients with scaphoid fracture with mean age of 32.5±7.94 years and mean duration of presentation after injury of 19.9 days (3 to 162 days).

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Table 1: Distribution of patients according to socio-demographic profiles and details of approach, MMWS and complication.

S no	AGE	SEX	SIDE	Herbert Type	Time To Surgery (Days)	Approach	Time To Union (Week)	MMWS (POINTS)	Remark
1	32	F	L	B2	5	Orif Volar	12	85	
2	34	F	R	B2	8	Percutaneous Volar	10	90	
3	45	M	R	B2	8	Percutaneous Volar	10	90	
4	22	M	L	B2	3	Orif Volar	10	95	
5	51	M	R	C	162	Orif Volar	14	80	Bone Grafting Was Done
6	36	F	L	A2	18	Percutaneous Volar	9	90	
7	28	F	R	C	44	Percutaneous Volar	11	70	
8	32	M	R	A2	14	Orif Volar	12	90	
9	33	F	L	A2	4	Percutaneous Volar	7	90	
10	21	F	R	C	56	Orif Volar	14	70	
11	21	F	R	A2	5	Orif Volar	12	85	
12	29	M	R	B2	8	Percutaneous Volar	12	95	
13	42	M	R	A2	5	Orif Volar	16	85	Cast Failure
14	42	F	L	B2	8	Orif Volar	16	80	Cast Failure
15	44	F	R	A2	4	Percutaneous Volar	10	95	
16	31	M	L	A2	1	Percutaneous Volar	8	100	
17	21	M	R	B2	12	Percutaneous Volar	12	90	
18	29	M	R	A2	5	Percutaneous Volar	8	95	
19	29	M	L	A2	16	Orif Volar	14	85	
20	41	M	R	B2	5	Percutaneous Volar	10	90	
21	23	M	L	A2	8	Percutaneous Volar	9	90	
22	32	M	L	B2	7	Orif Volar	14	85	
23	45	M	R	C	150	Orif Volar	14	80	Bone Grafting Was Done
24	31	M	R	B2	8	Percutaneous Volar	12	95	
25	24	F	L	A2	5	Percutaneous Volar	8	85	
26	34	F	L	B2	8	Orif Volar	14	80	
27	36	M	R	A2	4	Percutaneous Volar	10	95	
28	23	F	L	A2	1	Percutaneous Volar	8	100	
29	32	F	R	B2	12	Orif Volar	12	90	
30	31	M	R	A2	5	Percutaneous Volar	8	95	

In present study, 13 (43.3%) patients were operated within one week following injury whereas 2 patients were operated very late i.e. at 150 and at 162 days following injury. About 17 patients were managed with percutaneous fixation and rest 13 cases were managed using ORIF. Though, all the fractures treated either with ORIF or percutaneous fixation united successfully, delayed union was observed in 2 patients (6.7%) at 16 weeks postoperatively. Mean time for radiological union following ORIF was 12.6±2.4 weeks whereas that following percutaneous fixation was 9.2±2.5 weeks.

Range of motion was assessed using goniometer. Following percutaneous fixation ; wrist flexion averaged 61^0 (range 40 to 70^0) and wrist extension averaged 58^0 (range 35 to 65^0) whereas following ORIF, wrist flexion averaged 60^0

(range 40 to 65^0) and wrist extension 54^0 (range 30 to 60^0).

Modified Mayo wrist score (MMWS) was used to assess the final functional outcome. In present study, mean pain score was 22.4 (range 10 to 25) following percutaneous whereas it was 19.6 (range 10 to 25) following ORIF. Mean range of motion score according to MMWS following percutaneous was 24.3 (range 15 to 25) whereas that following ORIF was 22.3 (range 15 to 25). Mean grip strength score was 24.5 (range 15 to 25) after percutaneous fixation and 22.5 (range 15 to 25) after ORIF. Similarly mean activity score was 22.6 (range 15 to 25) and 19.4 (range 15 to 25) following percutaneous and ORIF respectively. The mean MMWS score was 93.8 (range 90 to 100) for percutaneous and 83.8 (range 70 to 95) for ORIF .

Delayed union was observed in 2 cases which were managed with open reduction with fixation. None of the patients showed malunion or signs of post traumatic osteoarthritis of the scaphoid or wrist at final follow up.

DISCUSSION:

The present prospective study aimed to assess functional outcome following ORIF or percutaneous fixation amongst 30 patients presenting with scaphoid fracture.

Injuries were graded according to Herbert and Fisher Classification which classify the fracture based upon fracture site and displacement. Clinical assessment at final follow up was performed using Modified Mayo Wrist Score (MMWS) which is a 100 point scoring system including 4 areas i.e. pain, range of motion, grip strength and activity comprising of 25 points each.

Mean age of the patients in present study was 32.5±7.94 years and slight male preponderance was observed in a ratio of 1.1:1. Brogan et al also observed similar findings in which scaphoid fracture were commonly encountered in young, adult males.[2] About 17 patients were managed using percutaneous fixation whereas 13 patients were managed using ORIF.

McLaughlin and Maudsley & Chen recommended ORIF amongst patients with scaphoid fracture to allow early mobilization of wrist,[17,21] and these findings were replicated by present study. In present study, union was observed in all the patients and the mean union time following ORIF and percutaneous fixation was 12.6±2.4 weeks and 9.2±2.5 weeks respectively. These findings were similar to study by Mittal et al in which mean union time following percutaneous fixation was 8.4 weeks (range 7 to 12) and that following ORIF was 12.1 week (range 8 to 16).[14] The benefit of percutaneous Herbert screw fixation is that the fracture reduction and fixation can be accomplished without further injury to the scaphoid blood supply.[22,23] Also reduced radiological healing time and less union time are the added advantage in fractures treated by percutaneous method.[14] Naranje et al also reported 100% union rate with Percutaneous Herbert screw fixation in 32 patients similar to present study.[24]

The present study observed union in 100% cases I both the techniques, but outcome using MMWS score including pain reduction, range of motion, grip strength and activity were significantly better in patients treated with percutaneous fixation as compared to ORIF. Though MMWS score was significantly better amongst patients treated with percutaneous fixation as compared to ORIF, complications in both the procedures were almost nil except for delayed union. The present study had certain limitations, i.e. small sample size and inhomogeneous population, the findings of the results could not be generalized.

CONCLUSION-

Since fracture treated with percutaneous fixation are associated with early union and early return to functional activity as compared to open reduction and internal fixation as replicated by MMWS score, Herbert screw for fixation with percutaneous technique for scaphoid fracture must be encouraged for displaced or undisplaced for better radiological and functional outcome.

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EFFECTIVENESS OF CAUDAL EPIDURAL STEROIDS IN DIFFERENT LUMBAR SPINE PATHOLOGIES

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ABSTRACT

Background- Epidural steroids are being used for the management of PIVD and central lumbar canal stenosis through different routes. Most common routes to administer epidural steroids for such patients include interlaminar, caudal and transforaminal. Controversy persists regarding the efficacy of epidural steroids in reducing the pain associated with various lumbar spine pathologies as well as controversy exist regarding the preferred route of injection.

Aim- The present study was done to assess the effectiveness of caudal epidural steroid injection in patients with various lumbar spine pathologies.

Methodology- This was a retrospective study on patients diagnosed as lumbar canal stenosis and PIVD and who received caudal epidural steroid injections in the last 6 months. Data regarding presenting complaints before epidural caudal steroid injection and improvement following the injection was obtained from all the patients. Back pain and leg pain were assessed separately using Visual Analogue Scale (VAS) and functional disability was measured using Oswestry Disability Index (ODI) before the procedure (assessed from records) and at the time of enrollment in the study. Statistical analysis- Data compilation was done with the help of MS Excel and data analysis was done using IBM SPSS 20 software.

Results- The present study included a total of 60 patients i.e. 30 patients diagnosed with lumbar canal stenosis (central and lateral) and 30 patients with PIVD (including multiple level PIVD). The mean VAS score and ODI significantly improved in cases of PIVD and lumbar canal stenosis but they were more effective in treatment of central lumbar canal stenosis and multiple level PIVD ($p<0.01$) as compared to other groups.

Conclusion- Caudal epidural steroid injections are effective, easy and safe method which can be conducted as a day care procedure. They may reduce the need of subsequent surgeries. Caudal steroid injections were more effective in treatment of central lumbar canal stenosis as well as multiple level PIVD.

KEYWORDS

PIVD, central lumbar canal stenosis, caudal epidural injections, ODI, VAS

INTRODUCTION

Low back pain is one of the commonest complaints amongst patients attending orthopedic clinic.[1] It has been estimated that approximately 80% of the population sustains an episode of low back pain (LBP) at least once during their lifetime with varying severity.[2] As the lumbosacral region is a critical area of spinal column which is subjected to greater forces as compared to other areas of the body, the prevalence of low back pain are high.[1] Also, low back pain is associated with sciatica in majority of cases. The etiology of low back pain includes lumbar disc herniation (prolapsed intervertebral disc), degenerative disc disease, lumbar canal stenosis, idiopathic etc.[3]

Prolapsed intervertebral disc (PIVD) is commonly diagnosed in MRI scans even in asymptomatic adults.[4] In PIVD, posterior longitudinal ligament gives way resulting in herniation of disc material into the spinal canal. It is characterized by acute-onset radicular or myelopathy-related symptoms on sudden exertion or lifting heavyweights.[5]

Another common cause of low back pain includes lumbar canal stenosis and is often due to multifactorial etiology. The factors contributing to lumbar canal stenosis include degenerative changes in the spine such as spondylosis, disc degeneration, facet arthropathy, and scoliosis etc. It is characterized by low back pain alone or with lower extremity pain, weakness or sensory changes aggravated by walking.[6] Despite the high prevalence of pain associated with lumbar canal stenosis and PIVD, the treatment remains controversial. Common treatments include conservative measures such as non-steroidal anti-inflammatory drugs (NSAIDS), activity modification, and physical therapy, whereas in a few cases, surgery is advised.[7] Epidural steroids are being used for management of PIVD and lumbar canal stenosis through different routes. Most common routes to administer epidural steroids for such patients include interlaminar, caudal and transforaminal.[8] Controversy persists regarding the efficacy of epidural steroids in reducing the pain associated with PIVD and lumbar canal stenosis as well as controversy exist regarding the preferred route of injection.[9,10] The present study was conducted to assess the effectiveness of caudal epidural steroid injection in patients with lumbar canal stenosis and PIVD.

Methodology

This study was conducted as a retrospective study on patients diagnosed with lumbar canal stenosis and PIVD and who received caudal epidural steroid injections in the last 6 months i.e. between 1st July 2019 and 31st December 2019. Inclusion criteria was all the patients belonging to age group of 18 to 60 years diagnosed as PIVD (including any level or multiple level) and lumbar canal stenosis (central and lateral) coming for follow up who were managed using caudal epidural steroid injections during the last 6 months in the Department of Orthopedics, People's College of Medical Sciences and Research Centre and People's Hospital, Bhopal. The exclusion criteria was patients treated with caudal epidural steroid injections for spondylolisthesis (as revealed by MRI). All the 60 patients i.e. 30 with PIVD and 30 with lumbar canal stenosis were selected using purposive sampling. The records of all the selected patients were also obtained from the MRD Department of our institute to supplement the information obtained from the patients. Data regarding sociodemographic variables such as age, gender, socioeconomic status was obtained from all the patients. Also, presenting complaints before the caudal epidural steroid injection and improvement following the injection was obtained from all the patients and entered in a questionnaire. Back pain and leg pain were quantitatively assessed separately using the Visual Analogue Scale (VAS) and the functional disability was measured using Oswestry Disability Index (ODI) version 2.0, before the procedure (from records) and at the time of inclusion in the study.

Statistical analysis-

Data compilation was done with the help of MS Excel and data analysis was done using IBM SPSS 20 software. Grouped data was expressed as frequency and percentage whereas numerical data was expressed as mean±SD. Paired t test was applied to assess the improvement in VAS and ODI amongst the patients. P value <0.05 was considered statistically significant.

RESULTS

The present study included a total of 60 patients i.e. 30 patients diagnosed with lumbar canal stenosis and 30 patients with PIVD.

Table 1- Distribution according to sociodemographic variables

Sociodemographic variables	Total (n=60)	Lumbar canal stenosis	Prolapse intervertebral disc
Mean age (years) Range (years)	45.1±9.6 18-60	46.9±7.8 31-60	43.2±11.3 18-54
Gender (M/F)	26/34	12/18	14/16
Weight	65.1±7.4	66.8±6.6	63.3±8.1
Height	158.6±43,7	157.3±44.2	159.8±43.1

The mean age of all the patients in present study was 45.1±9.6 years whereas that in patients of lumbar canal stenosis and PIVD was 46.9±7.8 years and 43.2±11.3 years respectively. Majority of patients in present study were females. (Table 1)

Table 2- Assessment of pain and functional disability in cases with Lumbar Canal stenosis

		Central Lumbar canal stenosis (n=17)	Lateral Lumbar canal stenosis (n=13)	P value
Visual Analogue Scale	Pre-intervention	7.89±0.36	7.65±0.8	0.14
	At enrollment	3.84±0.69	4.52±0.72	0.004
	P value	0.001	0.001	
Oswestry Disability Index (%)	Pre-intervention	61.1±2.8	59.8±7.7	0.43
	At enrollment	39.6±5.1	42.7±5.3	0.03
	P value	0.001	0.001	

Out of the 30 patients with lumbar canal stenosis, 17 patients were diagnosed as central lumbar canal stenosis whereas 13 patients had lateral lumbar canal stenosis. The mean VAS scores as well as functional disability amongst the participants with central and lateral lumbar canal stenosis before intervention was comparable in both the groups of lumbar canal stenosis. Though mean VAS scores as well as functional disability (ODI %) significantly improved in both the groups following intervention, the improvement was significantly higher in patients with central lumbar canal stenosis as compared to those of lateral lumbar canal stenosis ($p<0.01$). (Table 2)

Table 3- Assessment of pain and functional disability in cases with PIVD

		Single level PIVD (n=18)	Multiple level PIVD (n=12)	P value
Visual Analogue Scale	Pre-intervention	7.58±0.77	7.80±0.76	0.1
	At enrollment	4.11±0.53	3.96±0.71	0.001
	P value	0.001	0.001	
Oswestry Disability Index (%)	Pre-intervention	60.4±8.3	61.4±5.8	0.28
	At enrollment	43.3±5.1	40.1±3.4	0.01
	P value	0.001	0.001	

Out of 30 patients with PIVD, 18 patients were diagnosed as single level PIVD whereas 12 patients were diagnosed as multiple level PIVD. The mean VAS score as well as functional disability amongst participants with single level PIVD and multiple level PIVD before intervention was comparable in both the groups of lumbar canal stenosis. Though mean VAS scores as well as functional disability (ODI %) significantly improved in both the groups following intervention, the improvement was significantly higher in patients with multiple level PIVD as compared to those of single level PIVD ($p<0.01$).

DISCUSSION-

The utilization of epidural steroid injections for pain relief is practiced since 1952. They can be used as an invaluable non-surgical treatment for low-back pain radiating to the lower extremities.[11] Epidural steroid injections can be used by various approaches such as caudal, interlaminar or transforaminal.[3] All three routes are associated with certain advantages and complexities. The caudal route utilizes larger volumes of drug and is given away from the pathology site, but it is the easiest and safest route with minimal risk of dural puncture.[12,13] In the present study, caudal approach for administration of epidural steroid injections was used.

Though caudal epidural steroid injections are effective in the treatment of low back pain, the purported mechanisms of action remains unknown.[14] The steroids by their anti-inflammatory as well as immunosuppressant action along with membrane stabilization and inhibition of neural peptide synthesis help in reducing pain and improve outcome amongst patients with PIVD and lumbar canal stenosis.[15]

Ackerman et al in their study assessed the efficacy of lumbar epidural steroid injections amongst patients with lumbar disc herniations. They documented that pain score and functional score improve after 2 weeks of injections.[15] Singh et al in their study assessed the role of caudal epidural steroid injections in lumbar disc prolapse and documented significant pain relief in all the patients except one after 24 hours. However, symptomatic improvement was observed in 97.5% of the cases after 3 weeks whereas after 6 months, symptomatic improvement was seen in 67.5% of the cases.[16] These findings supported the findings of the present study i.e. significant improvement in pain as well as functional disability was observed in patients of PIVD following caudal epidural steroid injections.

Manchikanti et al also documented caudal epidural injections of local anesthetic with or without steroids to provide significant relief in patients with chronic function-limiting low back and lower extremity pain secondary to spinal canal stenosis.[17] Park et al conducted a study to assess the relationship between the severity of lumbar spinal stenosis using a grading system (grade 1 = mild stenosis with separation of all cauda equina; grade 2 = moderate stenosis with some cauda equina aggregated; grade 3 = severe stenosis with none of the cauda equina separated) and evaluated the response of patients with the help of short-term epidural steroid injections. They observed improvement (including reports of slightly improved, much improved, and no pain) in 78.7% patients at 2 weeks and 77.6% at 8 weeks after the procedure. However, they observed no association of degree of pain relief with grading of lumbar canal stenosis.[18] Statistically significant improvement in pain and functional disability was observed in cases of central lumbar canal stenosis following caudal epidural steroid injections in our study.

Literature suggests a varying success rate of epidural steroid injections. However, for a short term, maximum studies report a good success rate.[16] Choi et al in their study assessed the long-term benefits of epidural steroids in cases of LBP and studied its effect on pain, disability and need for subsequent surgery. The authors documented that ESI are effective for less than six months only.[19] In contrast, Sreen et al observed benefits of pain relief for 9 to 12 months after caudal ESI in patients with chronic back pain.[3] However, in the present study, we included the patients retrospectively i.e. those treated at least 4 weeks prior and no follow up was done and significant improvement was noticed for both pain as well as ODI in cases with multiple level PIVD and central lumbar canal stenosis.

Singh et al compared the efficacy of caudal epidural steroid injections with selective nerve block and documented >50% pain reduction till 6 months in selective nerve block group, while in the caudal group, >50% reduction of pain was maintained till 1 year. Similarly, the reduction in ODI in nerve block group was 52.8% till 3 months, 48.6% till 6 months, and 46.7% at 1 year, while in the caudal group, the improvement was 59.6%, 64.6%, 65.1%, and 65.4% at corresponding follow-up periods, respectively.[20] Similar results were documented by the present study in which mean VAS score as well as Mean ODI percentage improved significantly in cases with central lumbar canal stenosis and multiple level PIVD.

CONCLUSION

Caudal epidural steroid injections are effective, easy and a safe method which can be conducted as a day care procedure. They may reduce the need of subsequent surgeries. Caudal steroid injections were more effective in treatment of central lumbar canal stenosis as well as multiple level PIVD.

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Is in-vivo 80 N tensioned quadrupled hamstring graft better than conventional unmeasured pull for arthroscopic ACL reconstruction

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ABSTRACT

Purpose: To find clinical outcome of in-vivo standard 80 N tensioning of quadrupled hamstring graft during arthroscopic single bundle ACL reconstruction in comparison to traditional graft tensioning.
Methods: Sixty cases of isolated ACL tears were included in this study. All cases underwent Arthroscopic ACL reconstruction with Tibial attachment sparing quadrupled hamstring graft. Cases were divided into group I and group II (30 cases each). Graft Tensioning in group 1 was conventional one-handed unmeasured pull and in group II it was measured tension of 80 N with tensionometer during graft fixation. Pre-operative and post-operative (12 months) Anterior tibial translation (ATT) was measured with KT-1000 arthrometer. Clinical outcome was measured using Lysholm knee scoring system at 6weeks, 3months, 6months, 12 months and compared statistically among both groups.
Results: The mean pre-op ATT of 10.6 ± 2.04 mm (group I) & 10.83 ± 2 mm (group II) improved to 3.63 ± 1.16 mm (group I) & 3.63 ± 0.92 (group II) respectively at one year without significant difference (p value 1). The mean pre-op Lysholm score was 46.73 ± 6.77 (group I) and 45.97 ± 8.68 (group II). The mean Lysholm score at 6 weeks was 91.5 ± 2.78 (group I) and 93.43 ± 3.02 (group II) with significant difference (p value 0.014). At 3 months it was 95.4 ± 2.99 (group I) and 97.07 ± 2.07 (group II) with significant difference (p value 0.025). At 6 months it was 95.53 ± 2.46 (group I) and 97.5 ± 1.2 (group II) with significant difference (p value 0.0002). At 1 year it was 95.73 ± 2.22 (group I) and 97.8 ± 0.979 (group II) with significance (p value 0.0001).
Conclusion: The clinical score of ACL reconstruction is better when in-vivo 80 N tension is applied using tensionometer during graft fixation in comparison to conventional manual tensioning but there is no difference in ATT.

1. Introduction

Outcome of ACLR is dependent on many surgical variables and in vivo tensioning of graft at the time of fixation is an important one.[1,2]

Initial tension of graft can affect the clinical outcome of arthroscopic ACL reconstruction. If graft tension is inadequate then it may lead to knee joint laxity post reconstruction, which ultimately results in degenerative arthritis in long term.[3,4] If graft tension is in excess, then it may hamper the revascularization of graft and can result in graft failure. It can also lead to abnormal pressure on cartilage, meniscus and result in joint stiffness.[5]

In regards of optimal initial tension which could be given to graft at the time of fixation is still a matter of research, as till now optimum initial tension which would be required to obtain a stable and intact knee is not known. Some studies are in favour of low initial tension to graft, which may decrease the stresses within the graft, to avoid over constraining of knee as well as prevent frictional force of articular surfaces[2,6–8] whereas others are in favour of high tension to obtain a stable knee.[9,10] A graft tension of 20–90 N is supposed to be adequate but Arenja et al. reported 80 N to be an effective tension for ACL reconstruction.[10,11]

At present commonest method is one-handed pull technique. Manual feedback is used to determine the amount of tension applied to graft at the time of fixation. It is unmeasured initial tension but has generally produced good clinical results. The

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average initial tension used by orthopedic surgeons trained in sports medicine is 60 ± 29 N.[12]

There are studies suggesting no postoperative difference in clinical outcome or knee laxity between cases where measured tension was applied to hamstring grafts during fixation and conventional manual tensioning.[13,14]

There is paucity of literature that prospectively serially analyses clinical outcome of measured graft tension vis a vis unmeasured graft tension in ACLR with tibial attachment preserving hamstring graft.

The main purpose of this study was to find clinical outcome of in-vivo standard 80 N tensioning of quadrupled hamstring graft during arthroscopic single bundle ACL reconstruction in comparison to traditional graft tensioning. We hypothesize that 80 N of tension applied to quadrupled hamstring graft is effective to obtain satisfactory clinical outcome.

2. Methods

This prospective observational study was conducted between November 2013 to December 2015 at tertiary care center. An Institutional approval was obtained from the Ethics Committee before initiation of the study. Sixty matched skeletally mature cases with isolated symptomatic ACL tears were included in the study and were divided into two groups non randomly. In the first group I (30 cases) conventional unmeasured free one hand pull force was used for tensioning of graft and in group II (30 cases) tensionometer was used to apply 80 N pull force for fixation of graft. Cases with ACL and bony avulsion, associated with other knee ligament and meniscal injuries, fractures or cartilage lesion and osteoarthritis were excluded from the study. All cases were operated by the same surgical team. All cases underwent quadrupled single bundle tibial attachment sparing hamstring graft anterior cruciate ligament reconstruction by outside-in technique femoral tunnel creation method.[15] Detailed history and clinical examination were recorded in all cases. Magnetic resonance imaging (MRI) of the knee was done to document the diagnosis of ACL tear and other concomitant intra articular pathologies in all cases. Preoperative assessment was done using Lysholm's knee scoring system for functional assessment. Objective measurement of tibial translation (ATT) or anterior knee laxity was performed with KT-1000 arthrometer (MEDmetric, San Diego, CA) at manual maximum pull, being expressed as the difference between the injured and uninjured legs in 0.5-mm increments.

2.1. Operative procedure

Semitendinosus-gracilis tendons from the ipsilateral side were harvested with an open-loop tendon stripper from anteromedial incision on proximal leg and quadrupled graft was prepared keeping the natural tibial insertion of the tendon intact (Figs. 1 and 2). The diameter of the quadruple graft was measured.

A standard arthroscopic approach of the knee was performed through the anteromedial and anterolateral portals. After diagnostic arthroscopy, the femoral tunnel was created in an outside in fashion (Arthrex outside in jig) according to the graft thickness. The tibial tunnel was created with the help of a separate tibial guide. The quadrupled hamstring graft was then passed from the tibial tunnel to the external aperture of the femoral tunnel under arthroscopic vision. The proper seating of the graft on the tibia was checked and full knee range of motion was performed to rule out any form of impingement. The graft was tensioned at this stage by repeated cycling (20 cycles). A nitinol wire was passed through the femoral tunnel besides the graft and while maintaining a constant pull of 80 N using custom spring type tensioner along the long axis of graft for group II (Fig. 3) and free one hand pull force was applied in group I for graft tensioning. An interference (Biocomposite) screw (Smith- Nephew) was inserted with the knee in about 20° of flexion. Stability was checked by doing the Lachman and Pivot shift test after surgery. Patients in both groups were subjected to the similar postoperative protocol, which included early weight bearing with the help of crutches from day two of surgery. Active straight leg raise in all planes were started from day 2 after surgery. ROM knee brace was used for ambulation till the patient regained quadriceps control. At 6 weeks, 3 months 6 months and 12 months functional outcome was assessed by Lysholm knee scoring system. At 12 months anterior tibial translation was measured by KT1000 arthrometer. Proprioception was measured by active reproduction of passive movement.

2.2. Statistics

Preoperative and postoperative findings were compared using paired *t*-test. A p value of <0.05 was considered statistically significant. Statistical Package for Social Sciences (SPSS) version 21.0 was used for analysis. To compare anterior knee laxity the Mann-Whitney *U* test was used.

3. Results

In the present study thirty patients treated with an 80 N pull force for fixation of graft and thirty patients treated with the one hand pull were included. The two cohorts had similar baseline characteristics. The mean age of the patients of both groups was 29.83 ± 9.34 years in group I and 27.03 ± 7.71 years in group II which was comparable. The mean preoperative anterior tibial translation as measured by KT-1000 Arthrometer was 10.6 ± 2.04 mm in the group I and 10.83 ± 2 mm in the group II were also comparable (p value 0.667). There was significant improvement in the anterior tibial translation of affected knee at 12 months when comparing with its preoperative status in both the groups. The mean postoperative anterior tibial translation was 3.63 ± 1.16 mm in group I and 3.63 ± 0.92 mm in group II were similar (p value 1). Pivot shift test was negative in all cases of both groups at one year.

The preoperative Lysholm score was 46.73 ± 6.77 in group I and 45.97 ± 8.68 in group II was comparable between the two groups. However, mean postoperative Lysholm score at 6weeks was 91.5 ± 2.78 and 93.43 ± 3.02 for group I and II respectively which was statistically significant (p value 0.014). The mean postoperative Lysholm score at 3months was 95.4 ± 2.99 and 97.07 ± 2.07 for group I and II respectively which was statistically significant (p value 0.025). The mean postoperative Lysholm score at 6 months was 95.53 ± 2.459 and 97.5 ± 1.204 for group I and II respectively which was statistically significant (p value 0.0002). The mean postoperative Lysholm score at 12 months was 95.73 ± 2. and 97.5 ± 1.204 for group I and II respectively which was statistically significant (p value of 0.0001).

All the patients achieved quadriceps control in the form of sustained unassisted Active Straight Leg raising within 2 weeks Post-operative period (Table 1).

The mean follow up period was 16.5 ± 3.4 months in group I and 15.5 + 3.6 months in group II was comparable between the groups.

4. Discussion

In this study there was no case of graft laxity in either group despite allowing the patients to undergo accelerated rehabilitation protocol. None of the cases in either group reported of any instability till final follow up. There was no laxity objectively also, as KT-

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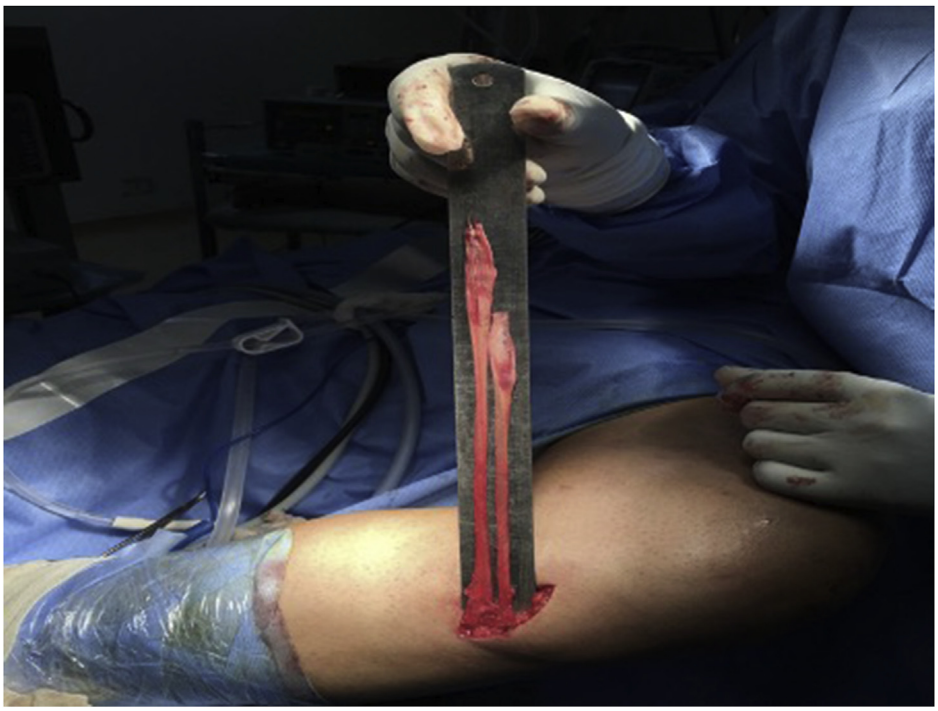

Fig. 1. Tibial attachment sparing harvest of Hamstring tendons.

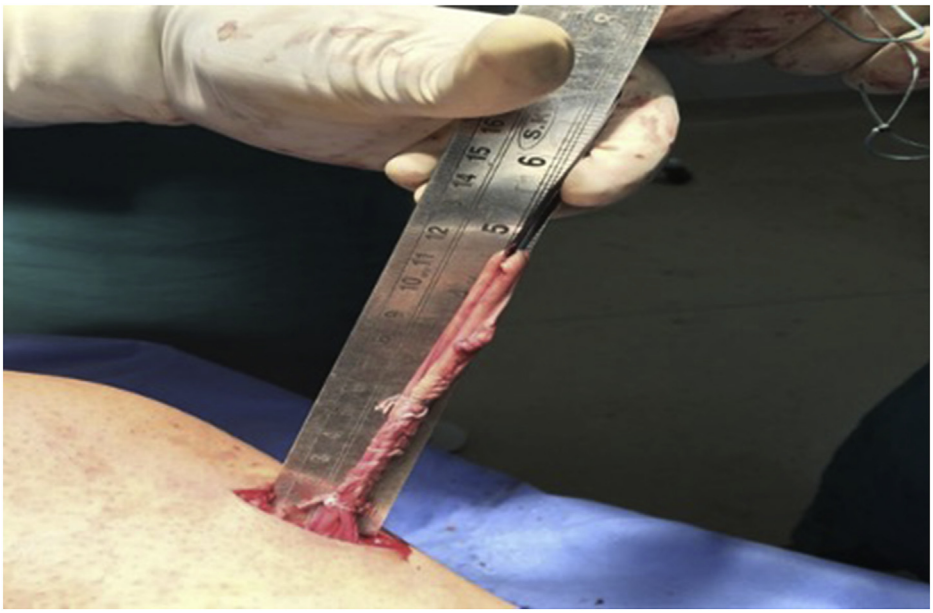

Fig. 2. Quadrupled hamstring graft.

1000 arthrometer showed a statistically significant reduction in anterior tibial translation (ATT) after ACLR along with negative pivot shift test. This finding supports the hypothesis that in-vivo 80 N tensioning of quadrupled hamstring graft is effective to obtain a satisfactory clinical outcome.

Tensioning of graft at the time of its fixation is one of the important surgical variables, which can influence the clinical outcome of ACL reconstruction. The tension of an ACL graft can be influenced by, diameter and graft length, type of graft, tunnel sizes of femur as well as tibia and accuracy in tunnel placement. In order to reduce variability, the surgical technique and postoperative rehabilitation protocol were standardized. Same team operated all cases by outside in technique from tibial footprint to femoral footprint graft placement. In order to reduce variability further, the same surgeon tensioned the graft in all cases. It is impossible to compare the graft tension with tension in native ACL of the uninjured side so the comparison was done using various parameters between pre-operative and post-operative status and also to the normal knee.

Arenja et al. reported 80 N to be an effective magnitude of

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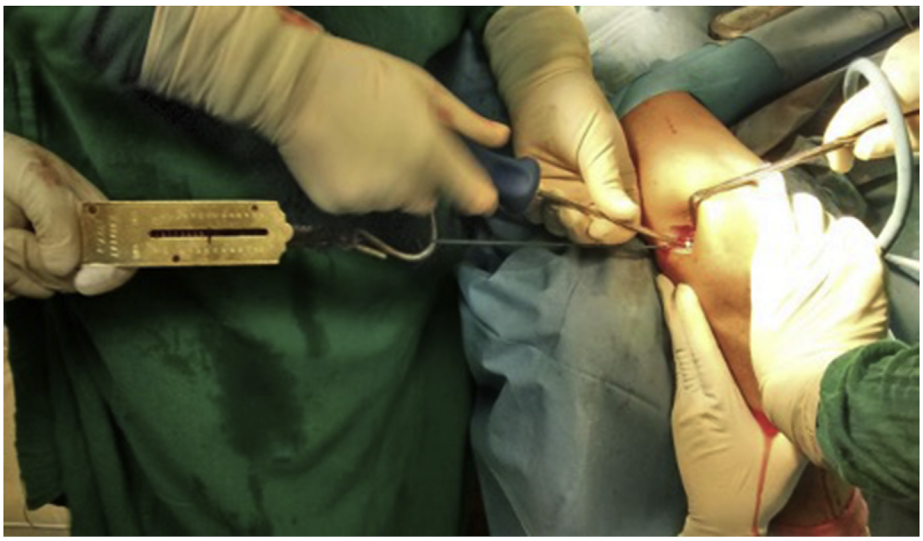

Fig. 3. Showing use of tensionometer to exert calibrated tension on hamstring graft during interference fixation on femoral side.

Table 1
Comparative clinical outcome of conventional and calibrated graft tension groups.

		Group I (Conventional method) n = 30	Group II (Tensionometer) n = 30	P value
Pre-operative	Mean ± SD	10.6 ± 2.04	10.83 ± 2.10	0.667
Anterior Tibial Translation	Range	8–14	7–14	
	Mean ± SD	3.63 ± 1.16	3.63 ± 0.92	1
Post-operative	Range	2–6	2–6	
Anterior Tibial Translation				
12 months				
	Mean ± SD	46.73 ± 6.77	45.73 ± 8.68	0.704
Pre-operative	Range	37–62	30–62	
Lysholm score				
Post-operative	Mean ± SD	91.5 ± 2.78	93.43 ± 3.02	0.014
Lysholm score	Range	86–98	87–100	
6 weeks				
Post-operative	Mean ± SD	95.4 ± 2.99	97.07 ± 2.07	0.025
Lysholm score	Range	91–100	91–100	
3 months				
Post-operative	Mean ± SD	95.53 ± 2.459	97.5 ± 1.204	0.0002
Lysholm score	Range	91–100	91–100	
6 months				
Post-operative	Mean ± SD	95.73 ± 2.22	97.8 ± 0.979	0.0001
Lysholm score	Range	91–100	91–100	
12 months				
Quadriceps control (days)	Mean	16.5 ± 3.4	15.5 ± 3.6	

tension for ACL reconstruction using hamstring polyester graft.[10] Yasuda et al. noticed a decreased laxity when 80 N tension was applied to the graft as compared to 20 N tension.[16] Similar finding in terms of ATT was reported by Nicholas et al. while comparing 90 N group (2.2 ± 1.6 mm) to 45 N group (3.0 ± 2.2 mm).[17] There is no benefit if graft tension is increased any further.[18]

A higher rate of failure with manual tensioning as compared to device-assisted tensioning has been reported[19] but we could not validate the same in this study as there was no case of graft failure in the manual tensioning group also. Because of the fact that most surgeons apply a variable graft tension of 20–80 N while tensioning manually,[20] it is wiser to use a tensionometer to apply calibrated and constant tension over graft.

If graft tension is more than the optimum, it will translate the femur anteriorly on to the tibia resulting in an over constrained knee, increase the joint reaction force and limit the range of motion of the knee. In this study there was no case of restricted range of motion in either group. All the cases could achieve full range of motion by 6 weeks.

Findings of this study are not in conformity with Grunau et al.,[14] who reported no difference in clinical outcome between measured tensioning of hamstring ACL grafts and conventional tensioning maneuver. The Lysholm score showed statistically significant improvement serially in both groups but it was significantly better in the calibrated tension group supporting its superiority.

With respect to the knee range of motion, there was no loss of extension or flexion in either group. In contrast Kondo et al. observed a significant loss of knee extension of 5–10° when 40 N tension applied to each graft at 30° of knee flexion in comparison to 30 N tension.[21] Post-operative quadriceps control was also obtained in the same duration in both groups.

4.1. Limitation of the technique

The graft preparation and arthroscopy are done sequentially not parallel therefore duration of surgery was slightly prolonged with

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mean tourniquet time of 54.73 ± 8.33.

5. Conclusion

Though calibrated tension of hamstring graft (80 N) in ACLR provides comparable stability and anterior tibial translation as traditional method of graft tensioning manually but has slightly better clinical scores. It has potential to improve outcome especially in early stage of learning curve.

Declaration of competing interest

None.

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Outcome after ORIF of intra articular calcaneal fractures without augmentation

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Abstract
Introduction: Calcaneum fracture is most common fracture in hind foot. In management of calcaneum fracture there is a lot of controversies either to use bone graft augmentation or not. But in this study we done Calcaneum plating without use of augmentation to reduce chance of infection, reduce graft site morbidity and reduce bleeding. In this prospective study we analyzed outcome of intra-articular calcaneum fracture treatment by open reduction internal fixation with calcaneum plate without any augmentation.
Material and Method: We selected 26 patients with intra-articular calcaneal fractures on basis of strict inclusion and exclusion criteria's from January 2016 to December 2016. All fractures were treated by calcaneum locking plate without any bone grafting, using the standard extended lateral approach. All patients were followed regularly at 2 weeks, 2 months, 3months, 6 months and 1year and outcome were evaluated by using American Orthopedic Foot and Ankle Society (AOFAS) Score.
Results: All fractures were healed with 84.6% of excellent to good results according to American Orthopedic Foot and Ankle Society (AOFAS) Score, only 2 patients had poor results. Radiologically average preoperative Bohler's angle was 6.8° and the average Bohler's angle at follow-up was 31.4°. At final follow up mean Gissane angle was 123.20 degree. Three patients (11.54%) developed chronic ankle pain on walking. Two patients (7.7%) were developed subtalar joint incongruity, two patients (7.7%) developed wound dehiscence, peroneal tendons irritation in one Patient and one patient developed Calcaneum Osteomyelitis.
Conclusion: Well Timed open reduction and internal fixation of intra articular calcaneum fracture with Locking Calcaneum Plate with respect of soft tissue envelope and early rehabilitation lead to therapeutic success. It does not required bone grafting or augmentation to early fracture healing.

Keywords: calcaneum plate, intra-articular calcaneum fracture, lateral extensile approach

Introduction
Calcaneum is the most common tarsal bone to get fractured. It account for approximately 2 % of all fractures, with displaced intra-articular fractures comprising 70–75 % of these injuries [1, 2]. Displaced intra-articular fractures carry a high morbidity and still a therapeutic challenge for orthopedic surgeons. Most common mechanism of calcaneum fracture is high energy axial load in falling. In such injuries, the lateral talar process driven into angle of gissane likes a wedge [3]. Plate osteosynthesis of the intra-articular fracture is a standard method of treatment. The first documented treatment of a series of calcaneum fractures with internal fixation was reported by Leriche [4] in 1922.
Ever since Lenormant first described the use of bone grafting to fill the space created after open reduction of a calcaneum fracture in 1928, this technique has maintained its popularity. However, the need for bone grafts in the treatment of intraarticular calcaneum fracture is still controversial, and there is no strong evidence to support any functional benefits of using bone grafts [5]. Surgeons in favour of bone grafting believe that it could stimulate fracture healing, leading to early full weight-bearing; may prevent posttraumatic arthritis; and could increase mechanical strength, thus helping to prevent significant late collapse [6, 7]. Those not in favour of bone grafts have stated that the highly vascular calcaneum heals radiographically in 4-8 weeks after surgery without bone grafting [8, 9, 10], Only internal fixation adequately support the articular surface and bone grafting increases the infection rate, blood loss, postoperative pain [11, 12], and they also consider donor site morbidity and complications involved with harvesting an autograft [13, 14]. We also believe that there is no need of bone grafting in treatment of intraarticular calcaneum fracture.
The purpose of the current study was therefore to evaluate the outcomes and complications of surgical treatment of intra-articular calcaneum fractures without any augmentation. We analyzed cases of displaced intra-articular calcaneum fracture treated by open reduction and internal fixation (ORIF) without bone grafts and observe the outcomes and complications in study group.

Aims and Objectives
Aim of this study was to evaluate outcome and complications after open reduction and internal fixation of intraarticular fracture of calcaneum without augmentation. We analyzed the infection rates, times to full weight-bearing, reduction of the

posterior facet, subtalar fusion rates, reduction of Bohler's angle, changes in the crucial angle of Gissane, changes in calcaneum height, and efficacy outcomes in study group.

Material and Method

This prospective and non-randomized study included 26 patients who were aged from ≥18 years and were diagnosed as having intraarticular calcaneum fracture. The fractures were stabilized with the open reduction and internal fixation with calcaneum locking plates by using extensile lateral approach without any augmentation at the authors' institutions from January 2016 to December 2016. All patients were selected on basis on following strict inclusion and exclusion criteria's.

Inclusion criteria: Patients(age ≥18 years) with Unilateral, displaced intra-articular calcaneum fractures (posterior articular facet step-off more than 2 mm, loss of height, and widening of the calcaneum, valgus deviation > 10°, varus deviation > 5°) of Sanders type II, III, or IV

Exclusion criteria were patients can treated conservatively due to patient's own choice or lack of surgical indication, associated fractures, lack of adequate skin condition, edema, and blisters in the lateral aspect of the foot, associated with other comorbidities, heavy smoking, refusal to undergo surgical treatment, bilateral fractures; and refusal to sign the inform consent form.

General physical examination, local examination, assessment of skin condition was recorded. Appropriate x-rays of ankle were taken for all patients (figure 1). All patients also underwent CT scan for further assessment of type of fracture and classified according to Sanders and Essex-lopresti classifications. All required laboratory tests were done in all patients for pre-operative fitness. Pre-operative counselling of the patient and his/her relatives regarding the method of treatment and prognosis was done and consent for surgery and for research study was taken.

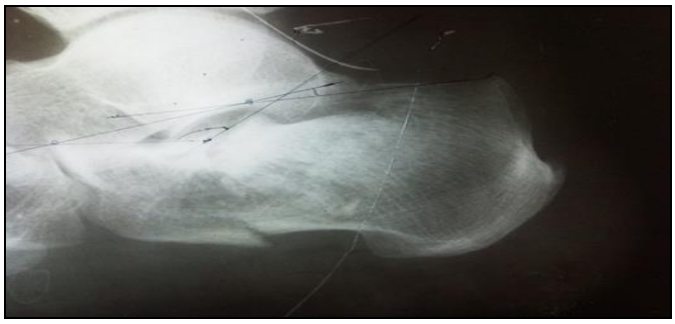

Fig 1: x ray lateral view of ankle showing calcaneum fracture with decreased bohler's angle

All patients were operated with the classic L-shaped lateral approach, incision starts from midway between the posterior region of the lateral malleolus and Achilles tendon, horizontal arm passing 3 cm below the lateral malleolus, extending to the calcaneocuboid joint. Due to the high risk of skin necrosis, dissection was made at the subperiosteal level. The flap was folded down and maintained cranially with three 2.0 mm K-wires attached to the talus, with visualization of the sheath of the peroneus muscles, which was preferably preserved. Under direct visualization of the fracture reduction was carried out, with K-wire temporary fixation performed after confirmation under C-arm intensifier. Definite fixation was made with calcaneum locking plate (figure 2). After closure by planes, an elastic compression bandage was applied with drain for 48 h and casting for four weeks. Partial load was authorized at the sixth postoperative week. Autografts to fill the space created inside the calcaneus were not used.

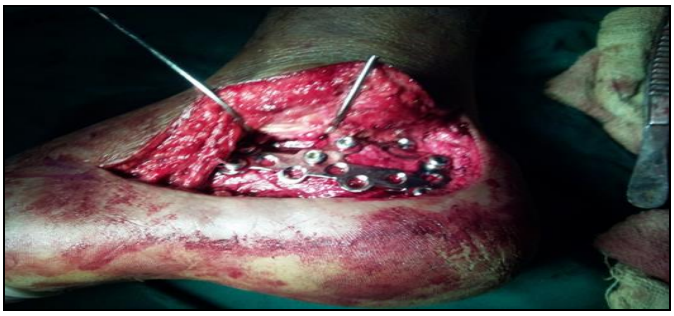

Fig 2: intra-operative image showing retraction with k-wire and plate placement.

All patients were followed regularly at 2 weeks, 2 months, 3months, 6 months and 1year. Outcomes were evaluated by using American Orthopedic Foot and Ankle Society (AOFAS) Score. Clinically, the following aspects were analyzed: subtalar joint in the standing and supine positions, varus and valgus deviation of the Hindfoot, Abduction, Adduction, Pronation and Supination of the forefoot, range of motion for ankle flexion and extension, appearance of surgical scars, and need for crutches. All patients underwent late postoperative radiographic (figure 3 and 4) assessment of Bohler's and Gissane angle and bilateral computed tomography with 5-mm thick axial, coronal, and sagital cuts.

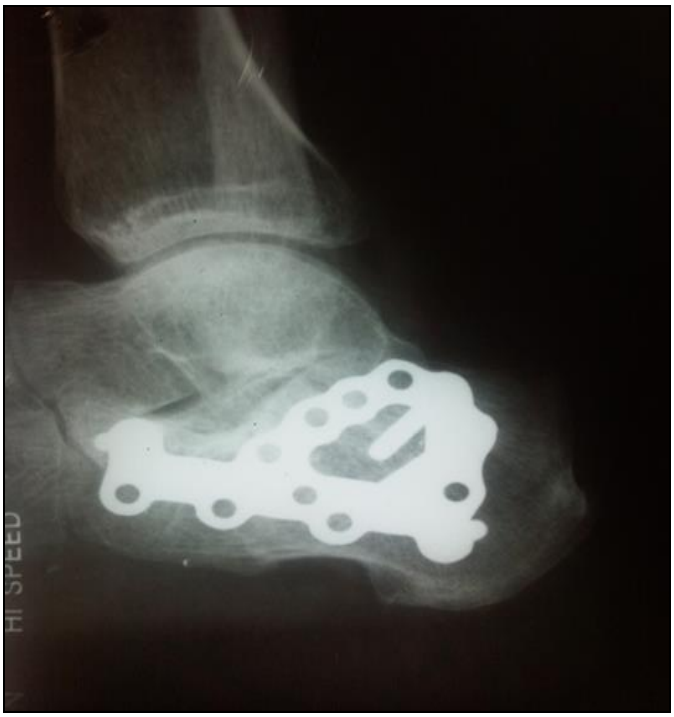

Fig 3: post operative x-ray lateral view calcaneum showing restoration of normal Bohler's and Gissane angle.

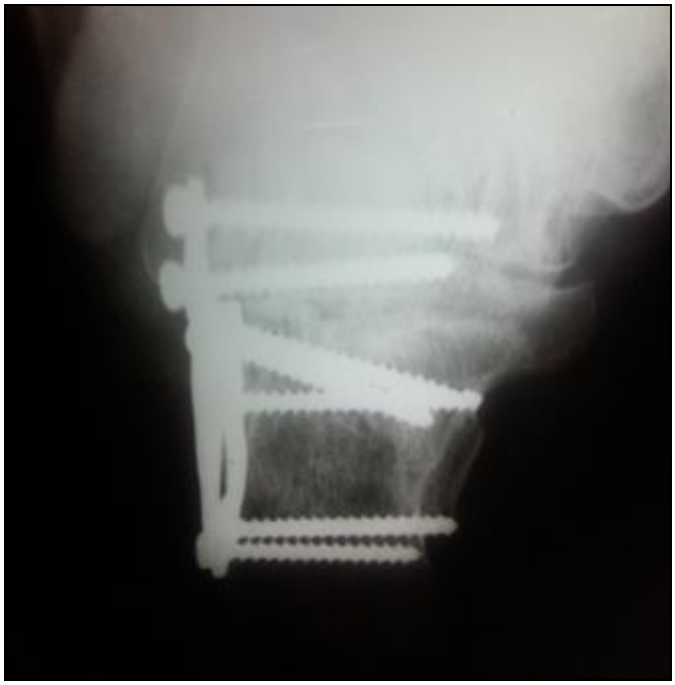

Fig 4: x-ray axial view calcaneum showing good reduction and no widening of calcaneum

Observation and Results

Among the 26 patients included in this study, 17 were men and 8 were women. Their average age was 42.6 (18–58).

The right side was involved in 19 cases and the left side in 7 cases.

The most frequent mechanism of injury was fall from height, occurring as an isolated fracture of lower limb. All cases were closed fractures.

None of cases included in this study were two-part shear fractures (type I). There were eighteen cases of type II fractures; four type IIA, eleven type IIB, three type IIC. Eight cases were type III fractures; five types IIIAB, two types IIIBC and one type IIIAC. Fifteen cases were classified as a joint depression type and eleven as tongue depression type. (TABLE 1).

Table 1

Sander's classification	No of patients
Type II	18
	Type II A=4
	Type II B=11
	Type II C=3
Type III	8
	Type AB=5
	Type BC=2
	Type AC=1
Essex-lopresti classifications	
joint depression type	57.7% (15/26)
tongue depression type	42.3% (11/26)

Surgery was performed after an average of duration of 8.21 days from admission (range 4–21 days).

There were thirteen excellent (50%), nine good (34.62%), two fair (7.7%), and two poor (7.7%). For simplicity, the excellent and good cases were rated satisfactory (84.62%). (TABLE 2)

Table 2

Excellent	90-100	13	50.00%
Good	80-89	9	34.62%
Fair	70-79	2	7.7%
Poor	<70	2	7.7%
Total		26	100%

Radiologically average preoperative Bohler's angle was 6.8° (range, −20° to 19°), and the average angle at follow-up was 31.4° (range, 20°–45°). This change represented an average restoration of Bohler's angle to 91.4% of normal (range, 67–100%). Similarly, calcaneum height showed an average correction to 92.6% of normal height (range, 85–100%).

The average residual widening of the calcaneum at follow-up was 9.2% of normal (range, 3–27%). The majority of patients had a congruent and non-arthritic joint, only two patients (7.7%) showed 2–3-mm step of articular surface and significant arthritic changes. At final follow up mean Gissane angle was 123.20 degree.

Pain at the lateral aspect of heel was the main complain of the majority of patients. Three patients (11.54%) developed chronic ankle pain on walking. Pain was tolerable and developed only after long walk. Two patients (7.7%) were complaints of Pain related to subtalar joint incongruity, two patients (7.7%) developed wound dehiscence, peroneal tendons irritation in one patients and one patients developed calcaneum osteomyelitis. Out of the twenty six patients included in this study, eighteen patients (69.2%) had mild swelling and eight patients (30.7%) moderate swelling. (TABLE 3).

Table 3

Complication	
Chronic ankle pain	3 patients (11.54%)
Subtalar joint incongruity	2 patients (7.7%)
Wound dehiscence	2 patients (7.7%)
Peroneal tendon irritation	1 patient (3.84%)
Calcanum osteomylitis	1 patient (3.84)
Mild sweeling	18 patients (69.2%)
Moderate swelling	8 patients (30.7%)

Twenty one patients (80.7%) returned to their previous occupation full time, while four patients (15.4%) attended the same job but with some restriction, and only one patient changed his job to a more sedentary one

Discussion

Calcaneum fractures are more common in young adult male those working on height and as young adults are economically active so these calcaneum fracture also have soioeconoic effect on society. In our study, we found that 65.38% of patients were male and 34.62% patients were females and had a mean age of 42.6 years. The right side was involved in 19 cases and the left side in 7 cases.

According to the literature, the most common cause of intra-articular fractures of the calcaneum is a fall from height, [15] which was confirmed in the present study, as this cause

accounted for 92% of the fractures.

The Essex-Lopresti [16] classification determines the line of fracture and allows treatment planning. According to the Essex-Lopresti classification, intra-articular fractures can be tongue-type or joint depression type. In most series, joint depression is the most frequent type of fracture, accounting for 43%–61% of intra-articular fractures [17, 18]. In our study, 57.7% of fractures were joint depression-type and 42.3%, tongue-type

Tomographic classifications help to assess the severity and prognosis of the injury; the Sanders classification is the most commonly used [19]. However, tomographic classifications are not uniform and each group aims to create its own classification, which makes it difficult to compare results as well as to identify the type of injury they describe. Tomography is considered to be an excellent test to identify details of the fragments and the joint impairment; however, it is not available in all services. This limitation justifies the use of a radiological classification.

In the last decade, open reduction and internal plate fixation of dislocated intra-articular calcaneum fractures has become a standard surgical method with low complication rate and better quality of life after the surgery. The method has been improved by implanting locking compression plates, the osteosynthesis is more stable, enables earlier weight-bearing, and bone grafting is rarely necessary[20,21]. In our study we used calcaneum locking plate in all patients and reported better functional outcome according to AOFAS Hind foot scale with better wound healing and less complications. We operated within first two weeks after injury because the surgery in the third week from injury is burdened with higher percentage of soft tissue healing complications and ORIF performed with more than three weeks delay is not recommended [22, 23].

The use of bone graft is controversial, some authors consider it to be osteoinductive and osteoconductive, while others consider it unnecessary [24, 25]. It is noteworthy that the use of bone graft increases the incidence of morbidity, as another incision is made for graft harvesting. In the present study, any bone grafts to fill the bone loss were not used. The lateral L-shaped access route has been widely used because it allows better visibility of the fracture, fragment reduction, and internal fixation and preserves the blood supply [24, 26]. In this study, the extended lateral L-shaped access was efficient; it was used as a standard technique for all cases. Wound necrosis is usually the result of improper incision and exposure for long surgery [27]. Necrosis is observed more frequently in the end of the lateral L-shaped incision [28]. In the present study, two patients needed surgical debridement due to skin necrosis, which solved the problem without the need of a skin graft. Symptoms associated with implants problems, which are rarely reported in the literature, include prominent implant, skin irritation, and heel pain. Problems usually arise because plate and screws cause irritation to the skin, tendons, or nerves, or because a screw penetrates the facet joint [29, 30] Tendon involvement due to implants can result in tendinitis or rupture, and lead to tendinitis and secondary pain [31]. In our study, no patients' complaints of implant related problems.

Assessing the results using the AOFAS scale, the literature presents rates of excellent results, ranging from 42.22% to 62% [32, 33, 34]. In this study, 84% of the results were considered good to excellent. It is not possible to state with certainty that the type of fracture may have influenced the score, as in the present sample, the number of tongue-type fractures was small when compared with joint depression.

Post-traumatic arthritis usually occurs in the subtalar and calcaneocuboid joints [35]. The literature reports an incidence rate of 1.2% in studies with long term follow-up [29, 36]. When intractable pain cannot be controlled by analgesics, subtalar arthrodesis may be the best option [29]. In the present study, two patients developed intractable pain due to subtalar arthritis and advised for subtalar arthrodesis to get relief from pain. In our analysis, we confirmed correlation between the Bohler's angle size and patient satisfaction in study group, as well as dependence of articular joint incongruence and the subsequent subtalararthrosis. This fact, proved and verified by a lot of other authors, confirms the role of Bohler's angle size as a predictive factor for subsequent late complications [22, 37]. Loucks in his prospective randomized study pointed out that initial negative size of Bohler's angle negatively influences postoperative results irrespective of therapy choice [5].

Conclusion

Well timed open reduction and internal fixation of intra articular calcaneus fracture with Locking Calcaneum Plate with respect of soft tissue envelope and early rehabilitation lead to therapeutic success. It does not required bone grafting or augmentation to early fracture healing. In general these fractures are operated with some delay. Considering the rare incidence of these fractures, need of special hardware equipment and relevant experience, the primary management of these injuries as well as complication treatment should be centered in specialized departments of orthopaedics or traumatolgy.

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Radiation Induced AVN of Hip Joint Following Pelvic Irradiation for Endometrial Carcinoma

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ABSTRACT

Introduction: AVN of the femoral head is a grave complication following pelvic irradiation. This case report provides an insight into diagnosis and management of this underestimated but important clinical problem .Due to its precarious blood supply, femoral head is one of the most common sites to undergo osteonecrosis following irradiation. A dose of 50 Gy has been accepted by various authors as threshold for development of radiotherapy induced AVN of femoral head.

Case: This is a case report of a 72 years old housewife who presented with localised pain in the right hip and groin for the last 2 months with no history of previous trauma or osteoarthritis right hip .On examination of right hip, patient had painful range of motion with 2cm of true shortening and 10 degree of fixed flexion deformity .In past Two years back, she was diagnosed as a case of adenocarcinoma of endometrium. She was treated by three cycles of chemotherapy followed by Abdominal Hysterectomy with Bilateral Salpingo-Opherectomy.Post operative she received adjuvant external beam radiotherapy and three sessions of intravaginal high dose rate (HDR)brachytherapy. She remained alright for two years following radiotherapy course .She presented to us with pain and limping right hip for last two months. Patient was subjected to detailed investigations like MRI, CT scan, Roentgenographic Evaluation and CT Guided Biopsy and was diagnosed as case of post irradiation AVN of right hip (STAGE IV). After having excluded the presence of metastases in the right hip, patient was taken up for cemented Total Hip Replacement with bone grafting of the medial deficient acetabular wall. Patient was discharged on 7th post OP day with full restoration of limb length and painless fully mobile hip without limping.

Conclusion: Post irradiation AVN of the hip joint remains a diagnostic dilemma for the clinicians and if not managed timely and adequately it leads to severe morbidity in the patients .A high index of suspicion and cautious exclusion of progression of skeletal metastases is the key in timely diagnosis of this otherwise rare but grave long term complication following pelvic irradiation.

Keywords: *Avascular Necrosis (AVN), Pelvic irradiation, Endometrial carcinoma, Bony Metastases*

INTRODUCTION

This case report documents post irradiation AVN right hip joint following the thereuptic pelvic irradiation for adenocarcinoma of endometrium .It is aimed in providing a valuable insight to clinician worldwide in the diagnosis and management of this underestimated grave complication. Pelvic radiotherapy continues to be an indispensible component in the treatment modalities of carcinoma of various pelvic organs namely endometrium, cervix, urethra, ureter and gonads .Inspite of revolutionary advancement in pelvic radiotherapy delivery protocols, AVN or Radiation Injury of hip [(1)] and its consequences continues to be the most severe and challenging long term complication.

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The term irradiation injury of hip coined by Duparc et al[1] and post irradiation osteoarthritis by Meary et al [2] signifies the hazardous outcome of pelvic irradiation on hip joint i.e. AVN. There are at least two postulated factors responsible for radiation induced AVN. Firstly, cellular component depletion caused by direct radiation insult [3].Secondly ,the local ischemia resulting from radiotherapy-induced micro vascular damage ranging from thickening of walls of blood vessels to their complete obliteration as reported by Mac daugall et al [4] and by Ewing J[5]. AVN most commonly affects the bones located distant from vascular territories especially those which have single terminal blood supply and limited collateral circulation such as femoral head, femoral condyle, head of humerus, capitulum and proximal part of the scaphoid and talus. [6, 7]

The critical dose above which osteonecrosis may occur ranges from 3000-4000 rads (30-40Gy).Apart from radiotherapy dose and irradiation volume , bone structure ,its location and patients age are relevant in determining the extent of damage .[8,9]

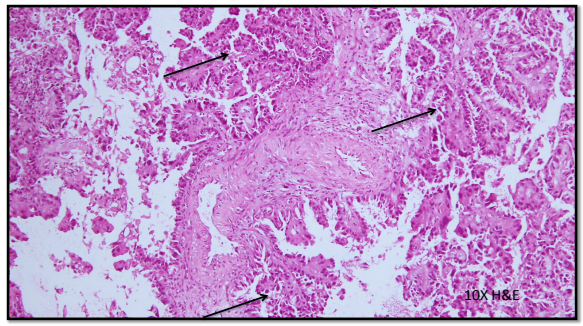
The treatment modality is determined by the stage of hip destruction at which patient presents. In most of the cases it is surgical which may range from core decompression in early stages to hip arthroplasty in advanced stages.

CASE

72 years old female, presented with pain in the right hip and limping for the last 2 months. She was apparently alright 2 months back when she started experiencing excruciating localised pain in the right hip .Pain got aggravated with movements and weight bearing and relieved by rest and analgesics. One month following pain patient started limping for which she required single stick for ambulation. She was systemically preserved with no history of trauma or osteoarthritis in the right hip.

Examination of the right hip revealed tenderness over the anterior joint line, painful range of motion, fixed flexion deformity of 10 degree, supra-trochantric true shortening of 2 cm and no other fixed deformities were present.

She has past history of frequency of micturation with burning sensation two years back for which she took gynaecology consult. On evaluation, she was diagnosed as a case of well differentiated adenocarcinoma endometrium (stage IV) (figure 1) and chronic nonspecific cervicitis. MRI revealed distended endometrial cavity with minimal residual tumour and no evidence of myometrium invasion.



ADENOCARCINOMA ENDOMETRIUM SHOWING PROMINENT PAPILLARY PATTERN (↗)

Figure 1: section of endometrium shows adenocarcinoma, having prominent papillary pattern .the papillae are lined by non mucinous, short columnar epithelium showing moderate nuclear pleomorphism

Patient was subjected to three cycles of chemotherapy. This was followed by total abdominal hysterectomy with bilateral salpingo-opherectomy with pelvic lymph node dissection with retroperitoneal lymph node sampling with total omentectomy under GA and sample was send for histopathology.

Histopathology report showed no viable tumour in right and left pelvic and retroperitoneal lymph nodes. Both parametria and omentum were also free of metastases. According to TNM staging tumour was staged as pT1N0MX.

Two months post operative X-ray Chest, CECT Abdomen and CA 125 were found to be within normal limits and she received adjuvant external beam radiotherapy using image guided external beam radiotherapy technique and three session of intravaginal high- dose- rate(HDR) brachytherapy (IVRT) for three consecutive weeks with dose of 7 Gy given at 1mm mucosa.

Following the radiotherapy course patient remained alright for next 2 years. She presented us two months back with history of severe localised pain and limping in the right hip. She was subjected to thorough physical examination and detailed investigations like Roentgenographic Evaluation (figure 2), MRI (figure 3), CT scan and CT Guided biopsy. After exclusion of metastases and with CA 125 within normal limits, she

was diagnosed as case of post irradiation AVN right hip. According to international classification of femoral head osteonecrosis (association research circulation osseous [ARCO]) and Ficat's classification, our patient was classified as AVN stage IV.

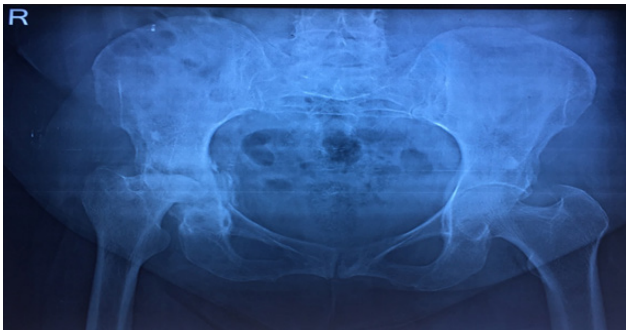


Figure 2: X ray pelvis with both AP: Osteolysis of femoral head and the acetabulum, Insufficiency fracture medial wall acetabulum with callus formation, avascular necrosis of femoral head- Flattening, sclerosis, deformity

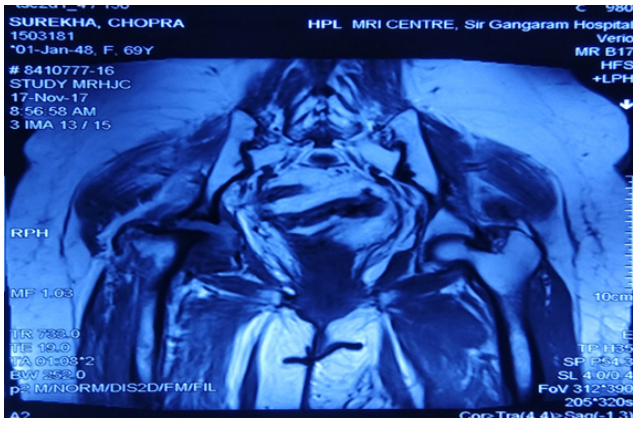


Figure 3: Complete resorption of the femoral head of the right side including the head and neck regions with fluid collection within the joint space and along the iliacus muscle and edema in the gluteal muscles. These findings are possibly post traumatic AVN.

Depending on patients age, her clinical status and AVN staging of the right hip she was taken up for cemented total hip replacement along with autobone grafting of deficient medial acetabular wall (figure 4). The capsule and part of deformed femoral head were send for histopathological evaluation which revealed radiation induced AVN changes and negated the presence of any granulomatous and neoplatic lesion (figure 5 & 6).

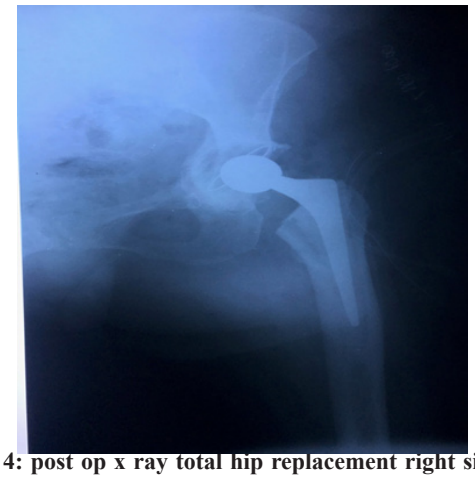


Figure 4: post op x ray total hip replacement right side with bone grafting over deficient medial wall acetabulum

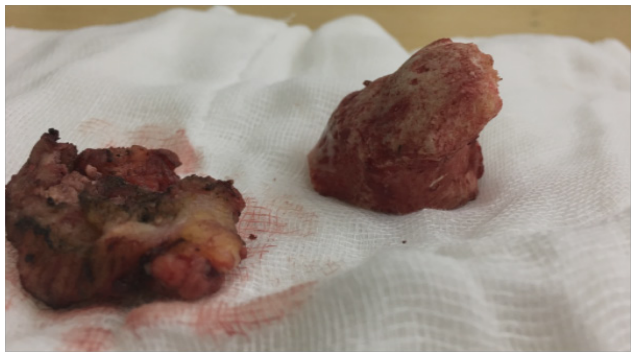


Figure 5: per operative specimen deformed head and capsule send for histopathology

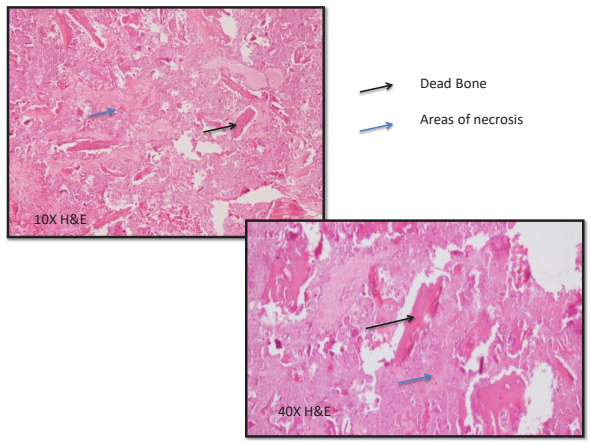


Figure 6: histopathology report of capsule of right hip joint and head of femur shows radiation induced AVN.

Patient was discharged on 7th post OP day with full restoration of limb length and painless fully mobile hip without limping.

DISCUSSION

Post irradiation AVN of hip joint is a late complication of previously irradiated pelvis. This is apparent from our case and by Hall FM et al [10] study was patients

developed AVN after 2 and 5 years respectively following pelvic irradiation. The long average latent period in the development of this complication emphasises the role of multiple etiological factors interplay however once they appear the disability progresses rapidly within a short span of time. In our patient, who was apparently alright 2 month back started experiencing excruciating pain in the right hip followed by gross limping.

Our patient received three cycles of cytotoxic chemotherapy pre operatively and post operative adjuvant external beam radiotherapy followed by 3 session of intravaginal HDR brachytherapy (IVRT) in the total dose of 50-54 Gy. As reported by Bragga et al [11] 50 Gy is the threshold dose to induce osseous necrosis. As postulated by various studies radiotherapy interferes with nutrition of the bone due to obliteration of vascular supply .This is supported by the fact that irradiation dose as low as 2500 rads initiates changes in the endothelium of the local blood vessels [12].Compromised circulation may lead to direct destruction of osteoblasts and impair their regeneration .The fine balance between bone formation and resorption is lost leading to deficient remineralisation, weakening and ultimately its collapse which are pathognomic of AVN changes. [13]

The radiation tolerance of the femoral head and neck is substantially lower than the long bones. According to the currently accepted normal structure tolerance guidelines, there is 5% risk of AVN if entire femoral head received 52 Gy which rises to risk of 50% after a dose of 65 Gy. With the modern times refined RT techniques and infrastructure , it is possible to minimise the post irradiation osseous damage of hip joint by delivering high doses to a limited volume with use of small irradiation field and protective shields which blocks the femoral neck and most of head. In situations where inguinal lymphnodes need irradiation, the femoral head and neck are invariably exposed to radiation dose above the threshold leading to osteonecrosis. [14]

In our case and in studies by different authors RT is not the only incremental causative factor to develop AVN of hip joint due to presence of other risk factors like concomitant administration of systemic cytotoxic chemotherapy ,Bisphosphonates, Bone Modifying Agents and long term androgen therapy .[15]

It is difficult to explain why our patient developed unilateral AVN of the right hip inspite of the fact that the left hip too received identical irradiation doses. As stated by different authors there may be a few contributory factors leading to progression of AVN changes in the hip joint. Firstly, Irradiation injury to the femoral head is severe in presence of involved inguinal lymph nodes requiring simultaneous irradiation. Secondly, there could be inadequate shielding of the involved hip as compared to opposite side. Last but not the least , it could be traumatic due to varying forces on one side owing to protective distribution of stresses due to altered biomechanics. This unilaterally increased stress is enough to cause collapse and fracture of the already vascular compromised bone [16].Weight bearing causes repetitive traumatic insult exacerbating and evolving picture of AVN.It is, therefore, pertinent on part of treating clinician to be aware of all the contributing factors leading to AVN of the hip joint in order to prevent progressive disability to the patient.

Depending on our patient's age, her severity of pain, limping and the extent of right hip destruction she was taken up for right hip cemented total hip replacement with autologus bone grafting of deficient medial acetabular wall. The surgical treatment remains the corner stone in management of AVN hip joint and preoperative staging determine the type of surgery. Core Decompression, Cortical Bone Grafting and Allograft procedure are advised in management of early stages .Whereas, Arthrodesis and Excision or Replacement Arthroplasty advised in management of late stages of AVN of the hip joint

High rate of acetabular failure has been reported by different authors in post irradiated THR.This has been explained by the increased mechanical insuffiency of the irradiated peri acetabular bone. Various authors have recommended metal re -enforcement rings to enhance the stability of acetabular component by improving the transmission of weight- bearing stress

from prosthesis to the bone .However, in our case we did not consider using of the metal re- enforcement ring because of relatively well preserved peri- acetabular bone.

CONCLUSION

Post irradiation AVN of the hip joint remains the diagnostic dilemma for the clinicians and if not managed timely and adequately it leads to severe morbidity in the patients .A high index of suspicion and cautious

exclusion of progression of skeletal metastases is the key in timely diagnosis of this otherwise rare but grave long term complication following pelvic irradiation. In recent times, with the revolutionary advancement in the total radiotherapy dose administration and protective shielding techniques, the incidence of post irradiation AVN of the hip joint and its resultant disability has been drastically reduced. The treatment modality is based on pre- operative staging and in most of the times it is surgical rather than conservative. Core Decompression, Cortical Bone Grafting and Allograft procedure are advised in management of early stages .Whereas, Arthrodesis and Excision or Replacement Arthroplasty is advised in management of late stages of AVN of the hip joint

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Conflict of Interest -NIL

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Role of high flexion total knee replacement in Indian scenario

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Abstract
This study was carried out between January 2016 & December 2016 to analysis the clinical and functional outcome of total knee replacement in Indian patients with high flexion posterior stabilized knee prosthesis. The range of motion (ROM) after total knee arthroplasty is an important component of patient overall functional outcome. The ability to the store posterior femoral translation has been shown be to an important factor in enhancing knee flexion after total knee arthroplasty. Knee society scoring and functional evaluation scoring system were used to collect clinic-radiological data. The range of motion (ROM) score preoperatively and 6 months postoperative was 19.30 (SD± 3.21); 96.5º and 24.53 (SD± 1.25) 122.65º respectively. The overall complication rate was 6.6. High flex knee improved patient abilities to perform activities that require weight bearing knee flex such as kneeling squatting and rising from sitting on the floor, activities very important in Indian scenario.

Keywords: high flexion knee, total knee replacement (TKR), range of motion (ROM), knee society score (KSS)

Introduction
The range of motion after total knee arthroplasty is an important component of a patient's overall functional outcome [1]. The inability of traditional prosthetic knees to consistently achieve flexion beyond 115 degrees and increased desire among patients to pursue activities associated with greater degree of knee flexion especially in Indian population as compared to western people as most of their routine habits and customs demand squatting (130°-full hip flexion and 111°-165° (or full) knee flexion), kneeling, or sitting cross-legged (90°-100° hip flexion and 111°-165° (or full) knee flexion [2, 3] have driven the development of knee prostheses designed to accommodate better and even facilitate higher degree of flexion [4]. A reduction in posterior femoral translation has been found to cause impingement of the posterior edge of the tibial component on the femoral shaft, thus preventing a high degree of flexion on the knee [5, 6]. The ability to restore posterior femoral translation has been shown to be an important factor in enhancing knee flexion after total knee arthroplasty [7]. High flex knee system is a refinement of the original standard design. It incorporate subtle changes in the geometry of the components to allow improved contact mechanics to address issues of wear and spin-out in the high-flexion ranges compared to traditional designs factors governing the flexion range of the knee after total knee arthroplasty.

Review of literature
Factors governing the flexion range of the knee after total knee arthroplasty include the degree of preoperative knee flexion, diagnosis leading to the operation, design of the prosthesis, surgical technique, and the patient's motivation to carry out rehabilitation. Although the mechanisms that hinder more flexion are unclear. Since Posterior Stabilized prostheses are characterized by highly excessive post against cam stress, lack of axial rotation, failure in providing full flexion and other problems thus such devices are also undesirable. The femoral component has a decreasing radius of curvature posteriorly and tibial polyethylene is free to rotate within the stem of the tibial base plate. The mobile bearing LCS system is versatile, and it may be used in both primary and revision arthroplasty. Pain relief and restoration of function have been very gratifying with this system. Despite good long term result reported in some series with the LCS knee prosthesis, there is controversy about the use of mobile instead of fixed bearings in total knee replacement. The Press fit Condylar Sigma posterior stabilized rotating-platform knee (PFC Sigma -RP) was introduced to improve the kinematics of the LCS RP prosthesis by employment of a post and cam mechanism in the PFC sigma PS-RP prostheses, would lead to consistent posterior roll back which in turn would lead to better knee range of motion, reduce polyethylene wear at the articular surface and provide better stabilization of the tibial insert. However no significant difference between LCS-RP and PFC sigma-RP has been reported. Current Buechell Pappas High Flex Knee System (3rd generation New Jersey device) is a refinement of the original LCS design. The anterior aspect of tibial polyethylene insert has been modified to reduce extensor mechanism impingement in high flexion, and optimization of cam-post design of Posterior Stabilized prosthesis to reduce the risk of dislocation in high flexion. Studies of bilateral TKA with a PCL-retaining prosthesis on one side and a PCL-substituting prosthesis on the other side have failed to show significant subjective performance or patient satisfaction

differences. The PCL causes the femoral condyles to glide and roll back on the tibial plateau as the knee is flexed this femoral rollback is crucial in prosthetic design. If the cruciates are excised, a more conforming tibial polyethylene component can be used to provide some degree of anterior and posterior stability. If the PCL is retained, the tibial surface must be flat or even posteriorly sloped. If a more conforming component is used in these circumstances, posterior impingement will occur. In multiple studies comparing PCL-retaining and PCL-substituting prosthesis, the average flexion attained at long-term follow-up has been similar. The loosening rates of these two designs are similar at 10-year follow-up, however, and, at least for the initial 10 to 15 years after surgery, this argument does not seem to be valid.

Materials & Methods

This study was done to know the clinical and functional outcome of Total Knee Replacement using Knee society score and functional scoring system with high flexion posterior stabilized prosthesis in the department of Orthropaedics at Sir Ganga Ram Hospital, New Delhi. 30 cases who met inclusion criteria underwent bilateral TKR using High-flexion Posterior-Stabilized Prosthesis were included in this study from January 2016 to December 2016. Patients were followed up post operatively for a period of minimum 6 months for evaluation of clinical and functional outcomes at 2 weeks, 6 weeks, 3 months and 6 months. All the clinic-radiological data was collected and registered in Performa as per the Knee Society Score and Knee Society Functional evaluation and scoring system.

Patient from either sex, Primary Osteoarthritis of bilateral knee, Thigh –calf index above 90º, BMI < 30, Age >60 years were included in this study whereas patients with Unilateral Osteoarthritis, Infective arthritis, Extensor mechanism dysfunction, Rheumatoid Arthritis, Neurological Disease, Revision TKR and ankylosis knee were excluded from this study. The analysis was carried out using Statistical package for social sciences 17.00 version. Normally distributed data was presented as means ± SD, or median (Range) if data skewed, and categorical data was presented as frequencies.

All patients were assessed clinically, radiographically (figure 1 and 2) and functionally using the Knee Society Score and Functional Score. Patients were made to lie in supine position with knee flexed to 90 degree. Under Tourniquet application sterile preparation was done from thigh to toes and draped. With the knee in 90 degree of flexion an anterior midline incision of 3 cm to 5 cm above the superior pole of patella was made and extended distally to below the level of the tibial tubercle. The retinacular incision was a medial parapatellar retinacular approach and patella was everted laterally. The degenerated femoral condyle was exposed. Appropriate soft tissue and ligamentous releases were performed prior to bone cuts. The extramedullary tibial guide was assembled using the adjustment screw at the ankle to align the resection guide keeping the long axis of the tibial resection guide parallel to the tibia. A stylus is used to check the amount of tibial cut. 2 mm for medial referencing, 10 mm for lateral referencing. The final tibial cut was completed with an osteotome to prevent over penetration of saw blade posteriorly which risked popliteal artery cut. Distal femur was resected with either the standard resection slot, which provides a 9mm resection from the prominent distal condyle, Distal Resection Guide and Valgus Alignment Guide were assembled onto the intramedullary alignment rod. The 5 to 7 degree valgus cut was made in order to get a distal cut that is perpendicular to the mechanical axis. Patella was denervated circumferentially using the cautery and patelloplasty done using a patellar clamp. Extension gap was checked with Trial Tibial Base. A symmetrical and rectangular extension gap must be obtained. A-P femoral sizer was placed flush against the resected distal femur and size adjusted so the feet contact the posterior condyles and the stylus contacts the shaft of the femur. Trial tibial base, equal in size to the femoral implant with the trial base handle and was placed against the proximal tibial surface. With the knee flexed, place the appropriate size femoral trial on the distal femur using the femoral impactor. Insert the trial tibial insert of equal size and appropriate thickness onto the trial base and complete the trial reduction. Bone cement was spread over the cut surfaces of the femur and tibia for femoral and tibial components implantation. Homeostasis is then obtained by sequentially removing the sponges from the lateral and medial sides of the knee, taking care to look specifically for bleeding from the superior lateral geniculate artery. The incision was closed in 3 layers over suction drain taking great care to close the elevated periosteal tissues to the patellar tendon. The knee was flexed past 90 degrees to ensure that no part of the closure limits flexion and that the patella tracks normally. The subcutaneous tissue and skin are closed with the knee in 30 to 40 degrees of flexion to aid in skin flap alignment.

The patients were assessed clinically, radiographically (figure 3 and 4) functionally (figure 5,6 and 7) using the Knee Society Score at an interval of 2weeks, 6 weeks, 3 months and 6 months post-operative.

Results and Observation

- In this study there were 30 patients with average age of 67.93 years (SD± 6.51). The mean ROM score preoperatively and 6 months postoperatively was 19.80 (SD ± 2.78); 99^0 and 24.33 (SD ± 1.37); 121.65 ^{0} respectively. The mean value of range of motion continued to increase up-to 6 month of follow up.
- Preoperatively no patient had flexion contracture of more than 20 degrees 80% of the patients (24/30) had extension lag of less than 10 degrees, 20% of patients (6/30) no extension lag preoperatively. Postoperatively, 17 patients at 2 weeks and 26 patients at 6 weeks had no extension lag, 28 patients had no extension lag at 6 month of follow up.
- The mean pain score preoperative and postoperative at final follow was respectively 9.67 and 40.85 according to the knee society knee score.
- The mean walking capacity score preoperatively and 6 months postoperatively was 26.67 (SD ± 7.30); and 47.33 (SD ± 4.66). The Walking capacity continued to increase up-to 6 month of follow up.
- The mean stairs function score preoperatively and 6 months postoperatively was 20.83 (SD ± 4.56); and 41 (SD ± 4.03).
- The mean preoperative and at 6 month KSS was

respectively 43.30 (SD ± 4.98) and 89.20 (SD ±5.0).

- The mean preoperative and 6 month postoperative KFS was 44.00 (SD ± 9.23) and 88.00 (SD ± 7.14)

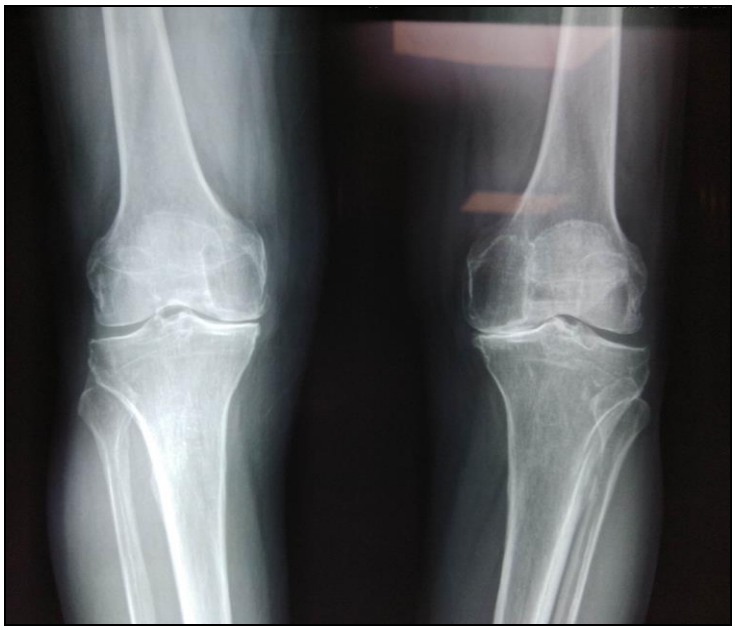

Fig 1: X-Pre Operative X Ray Bilateral Knee Ap View Weight Bearing

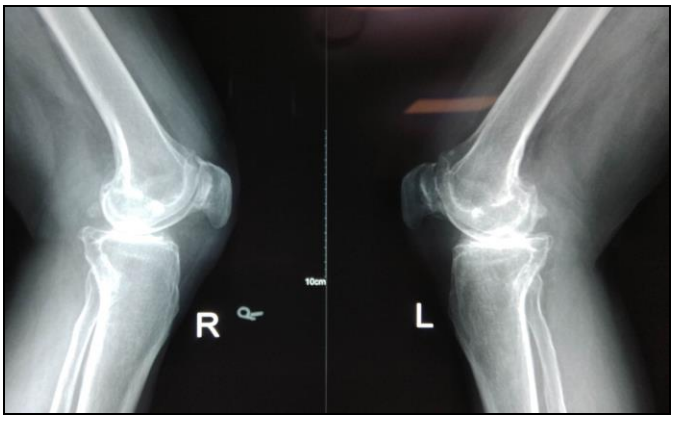

Fig 2: Pre Operative X Ray Bilateral Knee Lateral View

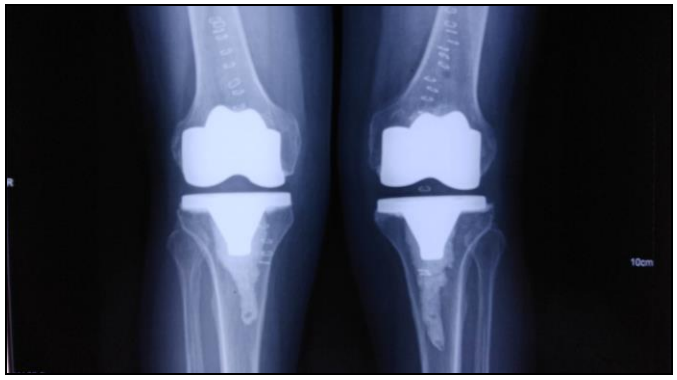

Fig 3: Post Operative X-Ray Bilateral Knee: Bilateral Total Knee Replacement (High Flexion Knee

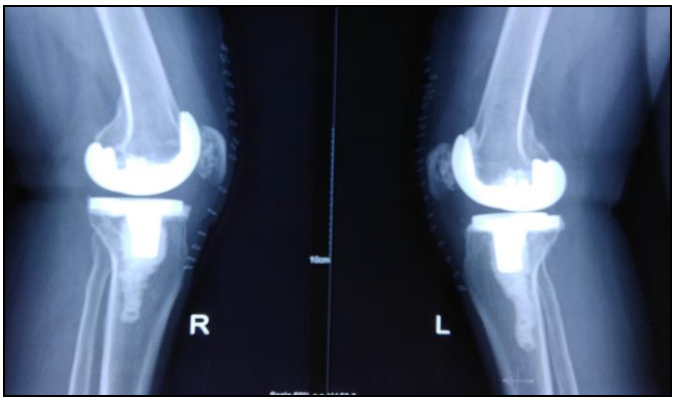

Fig 4: Post-Operative X Ray Bilateral Knee Lateral View

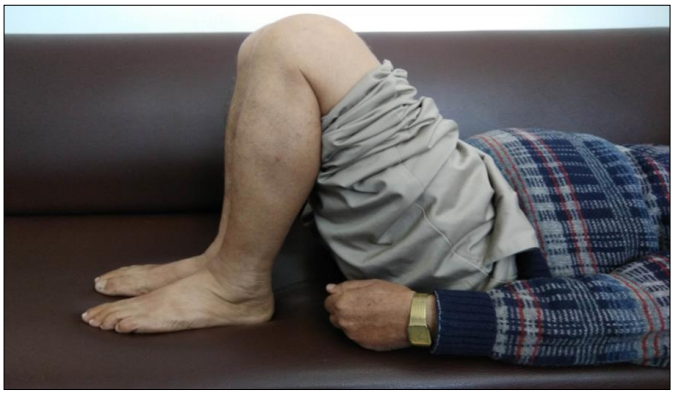

Fig 5: Flexion Up To 140 Degree Possible With High Flexion Knee

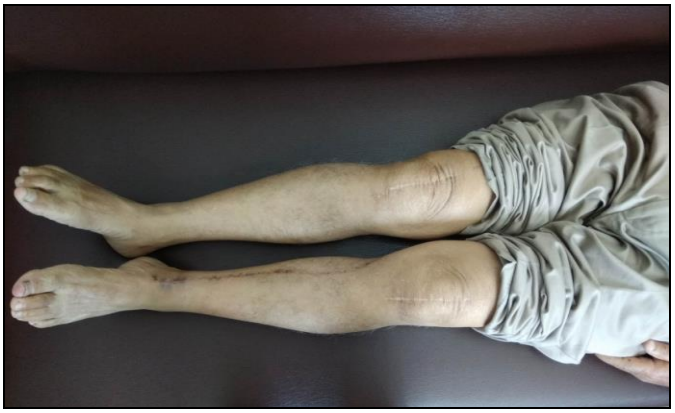

Fig 6: Varus and Valgus Deformity and Good Alingment after Total Knee Replacement

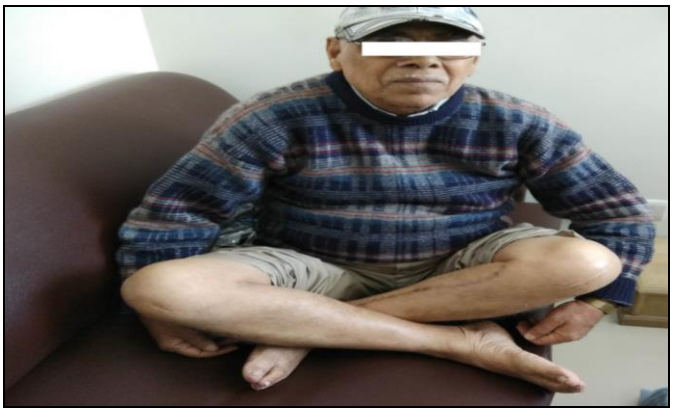

Fig 7: Follow UP Patient Can Do Squatting

Discussion

The High flexion design prosthesis is a modification of standard version posterior stabilized prosthesis. The modification is intended to allow deep knee flexion to be exerted safely and, hopefully, also lead to e better knee flexion range after total knee replacement. An additional 2 mm of bone is removed from the posterior condyle during preparation of distal femur. This allows an extension of articular surface in the high flexion design to facilitate deep flexion. The cam-post mechanism was modified to minimize the chance of posterior dislocation during deep flexion [8].

It is important to choose the correct femoral implant size and avoid using oversized femoral or tibial component [9, 10, 11, 12]. In our study 30 patients were taken meeting inclusion criteria. Post TKR follow upto 6 months all the patients had an improvement in knee function as assessed by the Knee Society and Knee functional score. The average gain in range of motion was 25.35^0 at 6 months follow up after TKR using high flexion prostheses. Steven H. Weeden MD and Robert Schmidt MD assessed 50 patients at 6 months and 1 year follow up after TKR significantly more patients had flexion greater than 135 degree in high flexion group than in standard group (P <. 05) [13]. Young-Hoo Kim, *et al*, study 50 patients received a Standard fixed bearing prosthesis in one knee and a High flexion fixed bearing knee prosthesis in the contra lateral knee. At the time of final follow up, mean ROM with High-flexion prosthesis was 138.6 degree. They found no significant difference between the groups with regard to ROM (P = 0.41) [14].

To compare the preoperative ROM and deformity to achieve final functional ROM, we divide the each variable into group like Stiff knee ($0\text{-}60^0$), Mobile knee ($61\text{-}90^0$), and Flexible knees ($>90^0$). 33.33% belongs to the mobile knee whereas rest (66.66%) belongs to flexible knee. In our study the over-all improvement in ROM was greater in knees with poor preoperative ROM because elimination of Flexion contracture contributed to the ROM. The mean preoperative and 6 month postoperative range of motion in Standard knee group were 84.5^0 and 115^0 degrees for the mobile knee, 105^0 and 124.5^0 for the flexible knees, compared to 82.5^0 and 119^0 degrees for mobile, 112^0 and 126^0 for flexible knee in High flexion knee group. Harvey *et al*. [15] observed that less mobile knees gained movement, but the more mobile knees lost mobility. McAuley, Harrer and Ammeen [16] assessed 21 patients with 27 stiff knees (< 50 degree ROM), out of which, 18 showed improved quality of life after total knee arthroplasty, as depicted by the increased walking tolerance, increased functional abilities, and decrease in pain. Mullen *et al*. in their study found little difference between the final post op ROM in comparing the stiff and the flexible knee groups with probable reason being small sample size and stiff knee being defined as < 90 degrees [17]. Pain relief was seen in all the patients irrespective of stiff, mobile or flexible knees and deformity. The overall complication rate was 6.66. Delayed wound healing was found in 1 patient. Complication rate was comparable to other studies. In our study there was no case of aseptic loosening of implants, deep infections, migration, synovitis, instability, extensive osteolysis and subluxation or dislocation of mobile bearing, as seen in other author's cases [18].

Our study had few limitations. First, possible limitation is that we measured the knee range of motion with the patients in the supine position, rather than under weight-bearing conditions. A second the knee scoring systems are prone to inter-observer variability and we have no inter-observer variability to ensure reliability. Dennis *et al*. [19] reported that weight-bearing ranges of motion differed significantly between high flex and standard TKR implants with similar passive non-weight-bearing ranges of motion. Nevertheless, the patients' abilities to perform activities that required weight-bearing knee flexion, such as kneeling, squatting, and rising after sitting on the floor. Third, accuracy of measurement of ROM of the knee with a clinical goniometer would be less than that compared with using an electro-goniometer or fluoroscopic guided radiographic measurement [20]. We recommend that long term studies of both clinical and functional outcomes are needed to determine the efficacy of high-flex knee prosthesis.

Conclusion

The high flex knee is a boon to patients in Indian scenario seeking increased function after TKR. It provides on an average 140^o of bending which allows activities requiring increased range of motion(ROM) like squatting sitting cross leg, kneeling, climbing and coming down the stairs and getting up from the ground.

High flexion knee is achieved through combination of prosthetic design, patient selection, surgical technique and special physiotherapy.

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Case Report

Case series of Ectopia Lentis associated with Marfan's syndrome

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Abstract

Purpose: *To report 4 cases of Marfan's syndrome with ectopia lentis.*

Methods: *Case 1- Fifty year old woman presented with sudden painless diminution of vision both eye since 1 day with history of blunt trauma by wood-log. Vision was counting finger 3 feet in both eyes, small pupil with sluggish reactions and posteriorly dislocated lens. B-scan showed posterior dislocation of lens in both eyes with retinal detachment in right eye. Case 2- Two sisters aged 15 and 12 year also presented with superotemporal subluxation of lens. Best corrected vision was 6/12 both eye in younger one and 6/9 in right and 6/12 in left eye of elder one. Case 3- A 22 year old male reported with painless diminution of vision since childhood. Vision was 1/60 in right and 6/60 in left eye. Anterior segment shows lens in anterior chamber in right eye and superonasal subluxation of lens in left eye. All patients presented with typical features of Marfan's syndrome like tall-statured with long, thin extremities; arachnodactly, prognathis and a high arched palate.*

Results: *Case 1 improved with +10 D lens in left eye and no improvement in right as there was Retinal detachment present. She was referred to vitreoretinal surgeon. Case 2 are under observation and managed conservatively. Case3 is also referred to vitreoretinal surgeon. Conclusion- Ectopia lentis is reported as most common feature in Marfan's syndrome. Though posterior dislocation is rarely reported. There should be multifaceted approach to manage such cases and prevent sight-threatening complication.*

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Keywords: *Marfan's, Ectopia Lentis, Visual Outcome*

Introduction

Marfan's Syndrome has been estimated to occur in 4 to 6 person per 100000 of population.[1] Ophthalmic features of this syn-drome have been frequently reported.[2] Ectopia lentis, the commonest ocular feature, occurs in 70 to 80 % of cases. Reports on isolated spontaneous complete poste-rior dislocation lying over the retina, or producing secondary complications like glaucoma or uveitis exist in litera-ture.[3] However, bilateral posterior dislocation is a rare feature. In our study we reported 4 cases of Marfan's Syndrome with ectopia lentis including case of post traumatic posterior dislocation lens.

Ectopia lentis is an acquired or hereditary condition in which lens is displaced from its natural position because of defects in the zonular filament. Karl Stellwag, an ophthalmologist from Austria, first formulated the term in 1856 and identified the lens movement within its normal space.[4] The Danish national survey carried out in 1993 showed that an estimated 6.4 per 100,000 individuals had ectopia lentis, of which mostly associated with syndrome.[5] Typically, ectopia lentis is associated with some acquired causes, such as trauma, inflammation, and hyper mature cataract. Although acquired causes are common, Williams postulated its genetic predisposition in 1875 and linked ectopia lentis to two generations in a family.[6] Genetic mutations, as in the case of Marfan syndrome (fibrilin1-gene), have been strongly associated with lens Subluxation with a rate of up to 60% of the cases because of a structural defect in the ciliary zonules.[7]

Case 1 :- A 50 year old woman presented with complain of sudden painless diminution of vision both eye since 1 day with history of blunt trauma over forehead. On ocular examination, vision was counting finger 3 feet in both eyes, slit lamp examination reveals normal cornea and anterior chamber, small pupil with sluggish reactions. Central fundus showed posterior dislocation of lens at 6 o' clock, rest details were normal. B-scan revealed hyperechoiec circular substance in both eyes, suggested posterior dislocation of lens. Also, separation between retina and sclera with clear subretinal space suggested retinal detachment in right eye. (Figure 1) Patient was improved to 6/9 with +10D in left eye

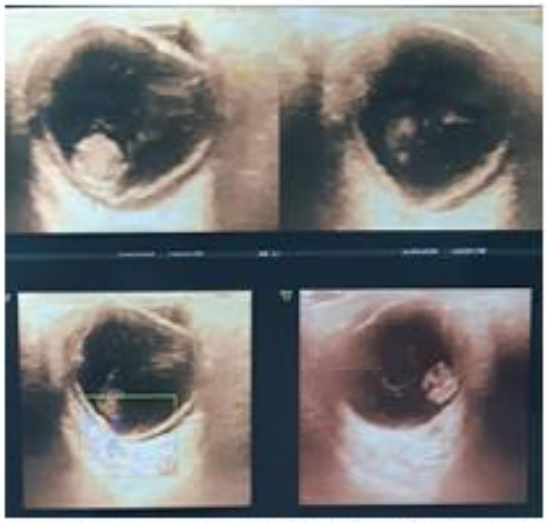

Figure 1: B-scan Picture showing retinal detachment with posteriorly dislocated lens

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Original Research Article

FNAC, cell block and core needle biopsy in diagnosis of lung masses: a necessity or choice?

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ABSTRACT

Background: Lung cancer is the commonest cancer mortality in the world. In targeted therapy era, precise cytohistological diagnosis is offered traditionally by FNAC, Cell Block (CB) and Core Needle Biopsy (CNB). However, little is known whether one technique is superior to other or all the three techniques complement each another. Therefore, this is a unique study as no other study has compared these techniques together till date. The objective of the study was to evaluate performance of FNAC, Cell block (CB) and Core Needle Biopsy (CNB) individually and comparing them with each other.
Methods: This was a prospective study of 50 cases who underwent two passes-1st for FNAC smears and Cell Block and 2nd for CNB.
Results: Material was Inadequate in 8 cases by FNAC 16 with Cell Block and 02 with CNB. When adequate, diagnosis and typing was possible by Cell Block (32) and CNB (48). In 08 FNAC cases having adequate material, cytological typing wasn't possible. These 08 cases were typed by cell block as 07 malignant and 01 pre-malignant. The combined inadequate cases with cyto-technique (FNAC and Cell Block) were 04 compared to 02 cases on CNB. Combined sensitivity of Cyto-techniques was 95.4% compared to 97% on CNB. The specificity was 100% for both Cyto-techniques and CNB.
Conclusions: Diagnostic adequacy and test parameters improved and approached CNB when both cyto-techniques are combined. So, we strongly recommend that Cell Block be made routine diagnostic procedure in all the government institutions especially for guided FNAC.

Keywords: Cell block, Core needle biopsy, FNAC, Lung masses

INTRODUCTION

Lung cancer is the most common cause of cancer-related mortality in the world. Its early and accurate diagnosis is the key for the optimal treatment.[1,2] In this era of targeted therapy, precise cytological and histological diagnosis is required.[3] This can be offered traditionally by FNAC, another cytology technique Cell Block (CB) and thirdly Core Needle Biopsy (CNB) which is competing these days with both the cytological techniques. Cell Block has an added advantage over FNAC of providing tissue architecture and use of ancillary techniques.[4] CNB on the other hand is a gold standard.

However, very little is known whether one technique is superior to other or all the three techniques complement each another.

A thorough search was done using 'FNAC', 'Cell Block', 'CNB', 'comparison' and 'Lung' as key words, but no study was found comparing all the three techniques together. Instead study comparing FNAC with Cell Block

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Clinico-Pathological Spectrum of Hansen's Disease at a Tertiary Care Center

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ABSTRACT

Introduction: Leprosy is an age old disease, described in the literature of ancient Indian civilizations in 6th century BC, but still it is a global burden especially in developing countries like India and Malaysia. The disease is caused by acid fast bacilli (AFB) *Mycobacterium leprae*, discovered by Hansen in 1874, with a characteristic neurotropism. Being a social stigma, its early detection based on clinico-histological features is mandatory. **Objective:** 1) Histological classification of clinically suspected new cases of Leprosy, according to Modified Ridley-Jopling classification. 2) To give Bacteriological Index (0+ to 6+) with Fite Faraco stain in every case. 3) To study age, sex, site distribution of leprosy cases. **Material:** Present study was a retrospective study conducted at a Tertiary Care Hospital over two years from June 2017 to June 2019. A total of 709 skin punch biopsies were obtained from Department of Dermatology and 222 were clinically suspected Leprosy cases. **Results:** A total of 222 leprosy cases were studied, out of which 141 (63.5%) were males and 81 (36.5%) were females. Majority of patients belonged to age group of 21 to 30 years. Most common type was BTT (50.9%), followed by LL (17.1%) and TT (9.5%). Least number of cases were found to be of Type II (ENL) reaction (0.5%). **Conclusion:** Leprosy is an "iceberg" disease, completely curable with MDT. Clinical collaboration of Dermatologist and Histopathologist is very important in accomplishing WHO's mission and India's National goal to **ERADICATE LEPROSY.**

Keywords: Leprosy, Ridley Jopling Classification, Bacteriological Index, Fite Faraco, Boderline Tuberculoid Hansen's, Histoid Leprosy

INTRODUCTION

Introduction: Leprosy is an "iceberg" disease. Leprosy is an age old disease, described in the literature of ancient Indian civilizations in 6th century BC. Leprosy or Hansen's disease is a chronic curable infectious disease mainly affecting cooler parts of the body like skin, mouth, respiratory tract, eyes, peripheral nerves and testes. Advanced disease may also spread to liver, spleen, bone marrow and kidney and may develop Amyloidosis. The disease is caused by acid fast bacilli (AFB) *Mycobacterium leprae*, discovered by Hansen in 1874, with a characteristic neurotropism. *M. leprae* is demonstrated in tissue sections, split skin and nasal smears by Fite Faraco (FF), Ziehl Neelson (ZN/ Acid Fast), Gomori Methenamine stains and Polymerase Chain Reaction (PCR). In tissue, organisms appear as "globi" or "cigar bundle".[1,2]

Clinically, the disease is characterized by hypopigmented patches, loss of sensation, thickened ulnar and popliteal nerves, erythematous/ maculopapular/ nodular lesions. They are multiple

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RESEARCH ARTICLE

COMPARATIVE STUDY OF FINE NEEDLE ASPIRATION AND CELL BLOCK TECHNIQUE IN SALIVARY GLAND LESIONS

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Salivary, Cell Block, Fine Needle Aspiration, Histopathology, Lesions

Abstract

Salivary gland lesions are relatively uncommon in head and neck region. Neoplasms of salivary glands are rare and have diverse histomorphological features in individual lesions and complex classifications. Differentiation of benign from malignant tumours makes these neoplasms diagnostically challenging. Fine needle aspiration cytology (FNAC) is a widely accepted diagnostic tool, due to the superficial location and easy accessibility of salivary glands. The Cell block(CB) technique is simple, reproducible and safe which retrieves small tissue fragments that are processed to form a paraffin block. Hence, the present study was undertaken to emphasize the role of combined approach of FNAC and CB technique in diagnosing salivary gland lesions. FNAC of 104 cases was done with CB's prepared from 99 cases and histopathological correlation was done in 34 cases. The sensitivity of FNAC and CB was 77.77% and 88.88% respectively while the combined sensitivity of FNAC and CB together increased to 100%. Thus, the combined use of both the techniques increases the diagnostic accuracy and helps the clinician in appropriate management of the patient.

Introduction:-

Salivary gland tumours account for about 3-10% of head and neck neoplasms[1]. The salivary gland system includes major and minor salivary glands. Parotid, submandibular and sublingual are the major salivary glands while minor salivary glands are present in the mucosal lining of the upper aero-digestive tract [2]. They are sites of origin of various non-neoplastic and neoplastic lesions. The non-neoplastic lesions include sialadenitis, sialadenosis and cysts. Neoplastic lesions can be either benign or malignant.

Ninty percent of these neoplasms originate from major salivary glands[3]. Salivary gland tumours affect the parotid gland in 70%, submandibular gland in 5-10%, sublingual gland in 1% and minor glands in 5-15% of cases[4]. Palate is the most frequent site of minor salivary gland tumours[5]. However, there are no reliable criteria to differentiate on clinical grounds the benign lesions from malignant lesions and morphologic evaluation is necessary. Neoplasms of the salivary glands are of particular interest to Histopathologists because of their varied histological and biological characteristics[6].

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Original Research Paper

Pathology

HISTOLOGICAL SPECTRUM OF LUNG LESIONS IN AUTOPSY CASES AT A TERTIARY CARE CENTRE

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ABSTRACT Autopsy study is of great diagnostic and educational value similar to the dictum "Mortui Vivos Docent". Millions of people around the world are being affected by numerous inflammatory, occupational and neoplastic conditions of lung. The aim of the present study was to analyze the histopathological spectrum of pulmonary lesions in all medicolegal and clinical autopsies. Retrospective data of 260 cases (Medicolegal and Clinical) was collected from postmortem reports over one year and cases with primary lung pathology as well as other cases where lung pathology was the secondary cause of death were studied. Present study showed overall male predominance (M:F=2:1). Maximum age group in present study was 31- 40 years. Most common pulmonary lesion in adults, children and newborn were pulmonary edema, interstitial and bronchopneumonia and Meconium Aspiration respectively. Thus, autopsy study in lung diseases forms the best educational tool to solve many diagnostic and legal discrepancies between Clinicians, Pathologist and Forensic experts.

KEYWORDS : Autopsy, Lung, Pulmonary Edema, Pneumonia

Introduction:

The word autopsy comes from the Greek word "autos – self, opis- view" which literally means "to see for oneself". It is synonymous to Post- Mortem(PM) and Necropsy.[1] Autopsy refers to self study of a dead body carried out for Medical, Legal or Scientific purpose.[1] Autopsy is mainly of two types: 1) Clinical/ Pathological- Done by Pathologist to know exact cause of death where legal consent of relatives is mandatory. 2) Medicolegal/ Forensic- Done by Forensic expert under the law of State for the protection of rights of citizens in cases of sudden, suspicious, unnatural or criminal deaths to establish the cause and manner of death. Here consent from relatives may not be required.[1] Thus, autopsy is carried out to establish the identity, cause, time of death, antemortem or post mortem nature.[2]

An autopsy includes detailed external examination and dissection of organs from cranial, thoracic, abdominal and pelvic organs.[1] These findings are further correlated and confirmed by histopathologist after microscopic examination of paraffin sections. Final cause of death is given by Forensic expert only after correlation with histopathological opinion. Thus, autopsy is a major guide to opine about the cause/ manner of death in both Clinical and Medicolegal autopsies. The importance of lung diseases in Pathology and Clinical Medicine is well known. Respiratory diseases have increased around the world due to air pollution, environmental inhalants and chemical toxins.[3] Millions of people around the world are being affected by preventable lung diseases.[4] Lungs are affected by numerous infections, inflammatory, occupational and neoplastic conditions.[5] Lungs are involved not only in primary lung diseases but also are invariably affected in all terminal events leading to death.[6]

Acute respiratory failure (ARF) is seen in 40-50% of overall deaths. Clinical and radiological findings in ARF are often non-specific.[7] In this scenario gross and microscopic findings in autopsy are a boon to arrive at cause of death.[7] Considering all the pros and cons of Post Mortem, bilateral lungs and pleural cavities should be thoroughly examined by Histopathologist and further correlate with clinical history and radiological findings.

The aim of the present study was: 1) To analyze the histopathological spectrum of pulmonary lesions in all medicolegal and clinical autopsies irrespective of the cause of death. 2) To study the primary pulmonary cause of death as well as all secondary changes in the cause of death from cerebral, cardiac, hepatic, renal or any other reason. 3) To describe the prevalence and pattern of respiratory infections, neoplasm and other non-communicable diseases in this part of Western Vidarbha, Maharashtra, India.

Materials and methods:

Present was a retrospective study conducted at Department of Pathology, GMC, Akola over a period of one year from July 2018 to June 2019. Retrospective data of 260 cases (Medicolegal and Clinical) was collected from postmortem reports. All autopsy cases irrespective of the cause of death were included. However, partial and completely autolysed specimens were excluded.

Original Research Paper

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Pathology

COMPARATIVE STUDY OF FLUORESCENCE AND AFB STAIN IN FNA SAMPLES OF EXTRAPULMONARY TUBERCULOSIS

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ABSTRACT The extrapulmonary tuberculosis (EPTB) is difficult to diagnose due to its pauci-bacillary nature. Aim of the present study is to do the comparative analysis of Fluorescence and Ziehl-Neelson stain upon FNA samples in clinically suspected cases of EPTB. **Methods:** Presentstudy was retrospective study of 100 cases of clinically suspected EPTB referred from Respiratory Medicine OPD. After procedure of FNA, smears were stained with routine H&E, PAP stain, ZN stain and fluorescence stain. Results were obtained after detailed study. **Results:** Out of 100 cases, presumptive tuberculosis was diagnosed in 54 cases showing either epithelioid cell granulomas or caseous necrosis or both upon morphology, while overall 26 cases were positive on ZN stain and 45 cases were positive on fluorescence. **Conclusions:** FNAC is the cheapest and simplest method to diagnose extrapulmonary tuberculosis, however those smears where TB cannot be diagnosed on FNAC like suppurative lesions, reactive lymphadenitis and low cellularity,fluorescence stain plays a key role for the correct diagnosis thereby significantly reducing the morbidity and mortality.

KEYWORDS : EPTB, FNAC, ZN, Fluorescence.

Introduction

Tuberculosis is one of the world's deadliest communicable diseases. Yearly, worldwide around 10 million people fall ill with TB according to Global Tuberculosis Report 2019 of World Health Organisation.1Annually, one fourth of the global incident TB cases occur in India and India has continued to top the list of TB burden.1,2Tuberculosis is caused by Mycobacterium tuberculosis and it commonly involves lungs but TB can affect any organ or system of the body. Extrapulmonary tuberculosis according to WHO classification criteria is an infection by M. tuberculosis affecting tissues and organs outside the pulmonary parenchyma.3,4In India, 10-15% of total TB cases are of extrapulmonary tuberculosis commonly affecting the pleura, lymph nodes, gastrointestinal tract and other organs with a significant mortality rate (25-50%).5Cytology and conventional smear microscopy have been used as the initial diagnostic tools for tuberculous lymphadenitis in resource poor settings.6Fine needle aspiration cytology is a simple and rapid diagnostic technique, but with low specificity due to common cytomorphological features between tuberculous and non-tuberculous cases.7,8Conventional smear microscopy lacks sensitivity due to the pauci-bacillary nature of fine needle aspirates (FNA).6Mycobacterial culture is a gold standard for diagnosis of TB and because drug susceptibility testings are not always available in resource poor settings, their results may take 4-8 weeks or even longer and it is expensive too.4Considering these limitations, more rapid and reliable methods like Fluorescence Microscopy are needed so that bacilli can be picked up within no time.

Koch first described the tubercle bacilli in 1882 which is now called as Mycobacterium tuberculosis. Mycobacteria are known to comprise a large group of acid-fast, alcohol-fast, aerobic or microaerophilic, non-spore forming, non-motile bacilli. 9However, the Ziel-Neelsen method for acid-fast bacilli plays a key role in the diagnosis and also for the monitoring of treatment in tuberculosis. Its major disadvantage is that it requires more than 5000-10000 bacilli/ ml turning to low sensitivity ranging from 20% to43%. 10, 11, 12Newer molecular techniques such as polymerase chain reaction (CBNAAT), although rapid, are costly to be routinely used in developing countries where most TB cases occur. 13Newer investigative methods like Fluorescent Microscopy plays an important role for detection of Mycobacteria because lower magnifications are used along with its less time consumption to examine the smears. Fluorescence microscopy using Auramine-Rhodamine (AR) or Papanicolaou (PAP) staining has been considered to be superior to ZN staining.14, 15The method is quick and inexpensive. The efficacy of autofluorescence and fluorescence in the diagnosis of extrapulmonary tuberculosis was evaluated for this purpose.

With this aim, the present study was carried out andthe results of FNA smear cytology were compared with ZN stain and Fluorescence microscopy findings.

Material and methods

Present studywasthe retrospective study conducted in the Department ofPathology, Government Medical College, Akola. All those clinically suspected cases of EPTB referred from Respiratory Medicine, ENT and Surgery OPDs were included in this study. So in total, we had included 100 cases. While those already diagnosed, recurrent and follow up cases of EPTB were excluded.

Procedure:

After obtaining detailed history and examining the patients, FNAC specimens were collected from 100 cases by performing 2-3 passes of 23-24 gauge needle attached to 5 ml syringe. Four smears were prepared from each aspirated material. Two were fixed with 95% isopropyl alcohol each for H & E and PAP staining. These smears were evaluated for adequacy and for the presence of epithelioid cell granulomas with or without caseous necrosis or only caseous necrosis. Third smear was for ZN staining. ZN stained smears were examined for bright pink beaded curved bacilli on bluish background and were reported as Positive/Negative for acid fast bacilli and the fourth one was for fluorescence microscopy. Smears for Fluorescence Microscopy were prepared with Auramine O stain which appears bright yellow fluorescent curved bacilli which were reported as positive and those cases showing similar fluorescence by crystals or fungal bodies were reported as negative.

Results

One hundred cases with clinical suspicion of tuberculosis subjected to FNAC, ZN stain and Fluorescence microscopy were studied. Most of the cases (27%) were found in the age group of 21-30 years with female preponderance(20%). The youngest patient was 4 year old male child and 88 years old male was the oldest patient. Overall, females (69%) showed predominance over males (31%) as shown in Table 1.

Original Research Paper

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Pathology

HISTOPATHOLOGICAL STUDY OF OVARIAN TUMORS AT A TERTIARY HEALTH CARE CENTER

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ABSTRACT **INTRODUCTION:** The ovary is a complex female genital organ in its embryology, histology and steroid-genesis and has a very high potential to develop malignancy at all ages. Ovarian cancers account for 3% of all female cancers .Ovary is the third most common site of primary malignancy of female genital tract preceded by cervix and endometrium.

AIMS: 1) To study the age incidence of different benign, borderline and malignant tumors. 2) To study the frequency of unilateral and bilateral tumors. 3) To study the frequency of positive peritoneal and omental secondaries in malignant conditions.

METHODS: Retrospective histopathological study of 200 cases of ovarian tumors was done over three years from January 2017 to December 2019 at a tertiary health care centre. Ovarian tumors were classified according to WHO classification.

RESULTS: Total 200 cases of ovarian tumors were studied with age incidence, youngest age was 2 years (Yolk Sac Tumor) and oldest was 80 years (Krukenbergs). Maximum cases were found in 4th decade (42%).Benign tumors were highest (66%), borderline (5%), malignant (27%). Surface epithelial tumors were commonest followed by germ cell tumors.

CONCLUSION: Ovarian tumors have a varied clinical presentation with respect to age, nature of origin and geographical distribution. High index of suspicion and early diagnosis with histological confirmation can significantly reduce morbidity and mortality.

KEYWORDS : Ovary, Ovarian tumors, Benign, Malignant.

INTRODUCTION

The ovary is a complex female genital organ in its embryology, histology and steroidogenesis and has a very high potential to develop malignancy at all ages. Few organs show a wide diversity of tumors like the ovary. Ovarian cancers account for 3% of all female cancers. Ovary is the third most common site of primary malignancy of female genital tract preceded by cervix and endometrium. Ovarian tumors exhibit a wide variation in structure and biological behavior. Ovaries are not clinically accessible and so easy screening methods for detecting ovarian tumors are not readily available[1]. Most of the ovarian cancers are detected when they have spread beyond the ovary thus accounting for a disproportionate number of deaths from cancer of female genital tract[2].A woman with an enlarging ovarian cancer will not be aware of its presence unless there is noticeable abdominal distention, pain, interference with bowel and urinary function , but by then there is high stage of that cancer . Thus, the outcome is poor and surgical treatment has to be aided by chemotherapy and radiotherapy. Early diagnosis is difficult due to asymptomatic nature ,inaccessible site and limited use of Ultrasound and CT guided FNAC .Not only for primary ,the ovary is favourite site for metastatic abdominal and breast cancers[3].Ovarian cancers has the worst prognosis among all gynecological malignancies .The overall 5-year survival rate is 45%,solely due to late stage at diagnosis .India being the second largest populated country of the world ,has large burden of the disease[4].

Advanced stage of disease at diagnosis, inappropriate management and poor compliance to therapy are all together responsible for the dismal survival rates[5,6] .The frequency ,clinical appearance and behavior of different types of ovarian tumors is extremely variable. In today's era of advanced chemotherapy and radiotherapy, the best therapeutic approach may be highly specific for a single type of neoplasm, hence clinical evaluation and accurate histopathological diagnosis are often critical factors in achieving a good spectrum of treatment response. Even today simple, cheap non invasive and reliable screening modalities are not available to diagnose ovarian tumors at earliest stage, thus morbidity and mortality is very high. In this scenario, accurate histopathological diagnosis of ovarian tumors by surgical pathologist remains a challenging task. However, over the past two decades, great advances in the knowledge about ovarian tumors, their molecular genetics, histopathologic features and immunohistochemistry have occurred and accordingly new therapeutic modalities have been established and are successful.

MATERIALS AND METHODS:

A Retrospective study of 200 cases of ovarian tumors was done over three years from January 2017 to December 2019, thus no ethical issues or consent from the patients was taken.

OBSERVATIONS AND RESULTS:

Total 200 cases of ovarian tumors were studied. All cases were studied with age incidence (table 1) youngest age was 2 years (Yolk Sac Tumor)and oldest was 80 years (Krukenbergs). Maximum cases were found in 4th decade (42%).Cases were also categorized in benign, borderline and malignant groups .Benign tumors were highest (66%), borderline (5%), malignant (27%). Size of benign tumors was less than 10cm and malignant tumors were more than 10cm in size.

Table no. 1: Age Incidence of Ovarian tumors (n= 200)

Age Range	No. of Cases			
	Benign	Boderline	Malignant	Total
1-10	4	0	6	10
11-20	10	0	5	15
21-30	40	1	6	47
31-40	75	4	8	87
41-50	11	4	13	28
51-60	6	0	0	6
61-70	0	1	4	5
71-80	0	0	2	2
Total	146	10	44	200

Clinical presentation at admission was studied in detail .Abdominal pain was the most common symptom (70%) followed by abdominal lump (50%), Gastrointestinal disturbances (20%), vaginal bleeding (18%), Ascites (12%), weight loss (10%), urinary complaints (8%). Parity status was also studied Ovarian tumors were most common in multiparous women (80%), primiparous (13%), nulliparous (7%).

Tumors were also categorized into unilateral & bilateral groups (table no.2).Benign tumors were unilateral (88.7%) & bilateral in (11.3%).All borderline tumors were unilateral (100%).Malignant tumors were unilateral in 75%, bilateral in 25 %.

Interestingly all bilateral malignant serous tumors (40%), showed

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Original Research Article

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Cartridge based nucleic acid amplification test: a sensitive diagnostic tool for tuberculosis on fine needle aspirates samples

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ABSTRACT

Background: The extrapulmonary tuberculosis (EPTB) is challenging to diagnose due to its pauci-bacillary nature. According to recent research, WHO recommends cartridge based nucleic acid amplification test (CBNAAT) to be used as initial diagnostic test in suspected cases of extrapulmonary tuberculosis. Aim of the present study is to assess the role of CBNAAT in comparison with cytomorphological features upon fine needle aspiration cytology (FNAC) and Ziehl-Neelson (ZN) stain in clinically suspected cases of EPTB.

Methods: Present pilot study is descriptive cross-sectional study of 439 cases of clinically suspected EPTB over a period of 12 months (January 2019 to December 2019). After procedure of fine needle aspirates, smears were stained with routine H&E, papanicolaou stain and ZN stain. In the same setting, aspirate was also sent for CBNAAT. Results were obtained after detailed study.

Results: Out of 439 cases, presumptive tuberculosis was diagnosed in 192 cases showing either epithelioid cell granulomas or caseous necrosis or both upon morphology, while overall 94 cases were positive on ZN stain and 146 cases were CBNAAT positive with the sensitivity of 84.04% and specificity of 80.57%.

Conclusions: FNAC is the cheapest and simplest method to diagnose extrapulmonary tuberculosis, however those smears where tuberculosis cannot be diagnosed on FNAC like suppurative lesions, reactive lymphadenitis and low cellularity, CBNAAT plays a key role for the correct diagnosis thereby significantly reducing the morbidity and mortality.

Keywords: Extrapulmonary tuberculosis, FNAC, CBNAAT, ZN

INTRODUCTION

Tuberculosis (TB) is one of the world's deadliest communicable diseases and worldwide around 10 million people fall ill with TB each year according to global tuberculosis report 2019 of World Health Organisation.[1] The causative agent is *Mycobacterium tuberculosis*. India stands first with the highest burden of TB.[1] Inspite of common involvement of lungs, TB can affect any organ or system of the body. Extra pulmonary tuberculosis according to WHO classification criteria is an infection by *M. tuberculosis* affecting tissues and organs outside the pulmonary parenchyma.[2,3] In India, 10 to 15% of total TB cases are of extra pulmonary tuberculosis which commonly involves the pleura, lymph nodes, gastrointestinal tract and other organs with a significant mortality rate (25 to 50%).[4] Cytology and conventional smear microscopy have been used as the initial diagnostic tools for tuberculous lymphadenitis in resource poor settings.[5] Fine needle aspiration cytology (FNAC) is a

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Clinico-Pathological Spectrum of Hansen's Disease at a Tertiary Care Center

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ABSTRACT

Introduction: Leprosy is an age old disease, described in the literature of ancient Indian civilizations in 6th century BC, but still it is a global burden especially in developing countries like India and Malaysia. The disease is caused by acid fast bacilli (AFB) *Mycobacterium leprae*, discovered by Hansen in 1874, with a characteristic neurotropism. Being a social stigma, its early detection based on clinico-histological features is mandatory. **Objective:** 1) Histological classification of clinically suspected new cases of Leprosy, according to Modified Ridley-Jopling classification. 2) To give Bacteriological Index (0+ to 6+) with Fite Faraco stain in every case. 3) To study age, sex, site distribution of leprosy cases. **Material:** Present study was a retrospective study conducted at a Tertiary Care Hospital over two years from June 2017 to June 2019. A total of 709 skin punch biopsies were obtained from Department of Dermatology and 222 were clinically suspected Leprosy cases. **Results:** A total of 222 leprosy cases were studied, out of which 141 (63.5%) were males and 81 (36.5%) were females. Majority of patients belonged to age group of 21 to 30 years. Most common type was BTH (50.9%), followed by LL (17.1%) and TT (9.5%). Least number of cases were found to be of Type II (ENL) reaction (0.5%). **Conclusion:** Leprosy is an "iceberg" disease, completely curable with MDT. Clinical collaboration of Dermatologist and Histopathologist is very important in accomplishing WHO's mission and India's National goal to **ERADICATE LEPROSY.**

Keywords: Leprosy, Ridley Jopling Classification, Bacteriological Index, Fite Faraco, Boderline Tuberculoid Hansen's, Histoid Leprosy

INTRODUCTION

Introduction: Leprosy is an "iceberg" disease. Leprosy is an age old disease, described in the literature of ancient Indian civilizations in 6th century BC. Leprosy or Hansen's disease is a chronic curable infectious disease mainly affecting cooler parts of the body like skin, mouth, respiratory tract, eyes, peripheral nerves and testes. Advanced disease may also spread to liver, spleen, bone marrow and kidney and may develop Amyloidosis. The disease is caused by acid fast bacilli (AFB) *Mycobacterium leprae*, discovered by Hansen in 1874, with a characteristic neurotropism. *M. leprae* is demonstrated in tissue sections, split skin and nasal smears by Fite Faraco (FF), Ziehl Neelson (ZN/ Acid Fast), Gomori Methenamine stains and Polymerase Chain Reaction (PCR). In tissue, organisms appear as "globi" or "cigar-bundle".[1,2]

Clinically, the disease is characterized by hypopigmented patches, loss of sensation, thickened ulnar and popliteal nerves, erythematous/ maculopapular/ nodular lesions. They are multiple

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Original Research Paper

Pathology

CYTOMORPHOLOGICAL SPECTRUM OF BREAST LUMPS: A ONE YEAR STUDY

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ABSTRACT Palpable breast lump is the most common clinical presentation. Breast cancer is the second most common cancer among women in India. The worldwide accepted protocol for diagnosis of breast lumps is the "Triple Assessment" a combined approach by the triad of clinical examination, mammography and FNAC. FNAC has a good sensitivity, specificity and accuracy in early diagnosis and management of breast lumps. The present study was carried out on 432 patients presenting with palpable breast lumps in the Cytopathology section over a period of one year from January 2019 to December 2019 with the aim of categorizing the breast lesions among females and males, studying age incidence & laterality. Fibroadenoma (44.9%), ductal carcinoma (11.8%), acute mastitis (6.4%) were the most common benign, malignant and inflammatory lesions respectively in the present study. FNAC for palpable breast lumps has proved to be an asset in the present study

KEYWORDS : Breast Lump, Fnac, Fibroadenoma, Ductal Carcinoma

INTRODUCTION:

Breast lump is the most common clinical presentation in most of the breast diseases. Breast cancer is the second most common cancer among women in India.[1, 2] The worldwide accepted protocol for diagnosis of breast lumps is the "Triple Assessment" a combined approach by the triad of clinical examination, mammography and pathological diagnosis by Fine Needle Aspiration Cytology.[3] FNAC is very reliable, safe, cost-effective and rapid method for diagnosis of breast lesions that can be easily carried out in outpatient department.[1,4,5] In this diagnostic era, a pathologist can perform many molecular ancillary techniques like estrogen, progesterone receptor and proliferation antigen on FNA samples and so, FNAC helps in deciding the proper therapeutic interventions for the breast lumps specifically in malignant lesions.[1] As most of the breast lumps are benign in nature, the preoperative cytological diagnosis can decrease the unnecessary surgeries thus reducing the morbidity.[6] FNAC has many benefits over breast lump biopsies.[1]

FNAC has a good sensitivity, specificity and accuracy in the diagnosis of both neoplastic and non-neoplastic breast lumps thereby assisting in early diagnosis and further management.[7] The present study was conducted to study the incidence and the various cytomorphological patterns of palpable breast lesions in patients by FNAC and subsequently to compare the results with those of other studies published in literature.

MATERIAL AND METHODS

The present study was carried out on patients presenting with palpable breast lumps in the Cytopathology section over a period of one year from January 2019 to December 2019 in Tertiary Care Hospital. A total of 432 cases were studied with detailed clinical history, clinical examination and imaging if available and consent was taken from all the patients. FNAC was performed by using 5 ml plastic disposable syringe and disposable 23 guage needles. Three slides were made from aspirated material, two were fixed with isopropyl alcohol and stained with Haematoxylene & Eosin and Papaniculou stains, while third slide was air dried and stained with May Grunwald Giemsa stain. In suspected cases of Tuberculous mastitis special stains like ZN and Fluorescence microscopy were also studied. FNA results were obtained after correlation with clinical history and radiological findings and then compared with findings of other studies in the literature.

RESULTS

A total of 2123 cases were obtained in the department of cytology over a period of 1 year between January 2019 and December 2019 out of which 432 cases (20.4%) were FNAC of breast lumps . All the 432 patients underwent a diagnostic FNAC in Cytopathology Section. In the present study, age range of patients varied from 9 to 85 years. Youngest patient (9 year old) was diagnosed with fibroadenoma and oldest (85 year old) was diagnosed with lobular carcinoma on FNAC. Majority of patients (28%) were noted in the age group of 21-30 years followed by the age group 31-40 years (24.5%) as shown in table 1.

Table 1: Age wise distribution of patients

Age group	Number of patients	Percentage
0-10	01	0.2
11-20	86	19.9
21-30	121	28.0
31-40	106	24.5
41-50	48	11.1
51-60	31	7.1
61-70	32	7.4
71-80	07	1.6
Total	432	100.0

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Original Research Article

Diagnostic Utility of Fine Needle Aspiration Cytology and Cell Block Technique in Thyroid Lesions

Authors

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Abstract

Introduction: *Thyroid diseases are among the commonest endocrine disorders worldwide. In order to avoid misdiagnosis and hence unnecessary surgery in patients with benign thyroid nodules, it is essential to distinguish between benign and malignant lesions. Despite the proven clinical importance of thyroid FNAC, it still has limitations. To overcome these limitations to some extent Cell Block technique can be used. It has a pivotal role in diagnosis and ancillary studies. In this study, we evaluated the role of Cell Block as a useful adjunct to cytological smears for establishing a more definitive Cytopathological diagnosis.*

Material and Methods: *Present study was a prospective cross sectional study conducted from Nov 2017 to Oct 2019. 334 cases with thyroid swelling underwent FNAC. Of these 334, Cell Blocks were prepared by plasma thromboplastin method for 273 cases and histopathology was available for 66 cases.*

Results: *In this study, the age range was 8-76 years. Maximum number of cases were 96 (28.75%) in age group of 31-40 years. Female to male ratio was 10.1:1.The Sensitivity, Specificity, Positive predictive value, Negative predictive value and Diagnostic Accuracy of Cell Block in categorising neoplastic and non-neoplastic thyroid lesions was more than FNAC and when these two techniques were used together all statistical indices increased further.*

Conclusion: *Cell Block is a complimentary technique to cytological smears to increase the Sensitivity, Specificity and Diagnostic Accuracy in thyroid lesions.*

Keywords: *Thyroid lesion, FNAC, Cell Block, Adequacy, Diagnostic Accuracy.*

Introduction:

Thyroid diseases are among the commonest endocrine disorders worldwide and in these diseases early diagnosis and treatment remain the cornerstone of management[1]. The thyroid swelling may be diffuse or nodular; may be solitary or multi-nodular. However, solitary nodules are more cause of concern due to the possibility of the nodule being malignant[2].

Aspiration cytology as a routine diagnostic technique in diagnosis of palpable lesions of various organs was introduced by Martin HE and

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Original Research Article

Histopathological Patterns in Abnormal Uterine Bleeding at a Tertiary Hospital

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Abstract

Background: Abnormal uterine bleeding (AUB) is related with patient's social,familial and personal life because of the considerable morbidity it causes. The aim of the study was to examine the histopathological patterns of endometrium in patients presenting with AUB as well as to find out its incidence in different age groups.

Materials and Methods: This is a retrospective study, conducted in the Department of Pathology, in a tertiary care teaching hospital, Udaipur from Jan. 2016 to Jan. 2018. The study includes cases of AUB with a probable endometrial cause.The study was done in the form of measures and percentages and showen as tables where found necessary.

Results: A total of 215 cases were studied.The age of the patients were from 18-72 years. Incidence of AUB was most common in the perimenopausal age group. Menorrhagia was the most common presenting complaint. Proliferative endometrium was the most common histopathological finding and was seen in 35.34% patients, followedby secretory endometrium in 18.60% patients, and disordered proliferative endometrium in 17.67% patients.Hyperplasia was seen in 16.73% cases. Malignancy was detected in 2.36% of cases and all were endometrial carcinoma.

Conclusions: Themain indication of endometrial biopsies is to rule out malignancy and endometrial hyperplasia in women over 35 years of age group. When no organic pathology was found,different physiological patterns like secretory phase,proliferative phase and other endometrial changes were observed. The most common endometrial pathology observed was proliferative phase endometrium.

Keywords: Abnormal Uterine Bleeding; Dilatation and Curettage; Endometrium.

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Introduction

Most common tissue specimens received inthe pathology laboratory are endometrial biopsies and curretings. The surgical pathologist faces a unique challenge due to different aspects of these specimens.As the cyclical hormonal influences and pregnancy affects uterine growth, the endometrium undergoes different types of morphologic changes especially during the reproductive years. These morphologic changes are complicated by the biopsy induced artifacts.

Abnormal uterine bleeding is the most common cause for performing an endometrial biopsy. AUB is the term that refers to any nonphysiologic uterine bleeding. Causes of abnormal uterine bleeding differ according to the age and menstrual status of the patient so these two parameters are very important data for analysis of the causes of bleeding [1].

Anovulation, adenomyosis, polyps and fibroid are the important causes of AUB. Endometritis, hyperplasia, disordered proliferative endometrium, cyclic endometrium,

Accuracy of fine needle aspiration cytology in categorization of breast lumps

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Abstract

Diagnostic breast lump FNAC is an important part of of triple assessment. At a tertiary hospital retrospective study was done.The study compared malignant lesions diagnosed on fnac with its histopathological diagnosis. 125 cases were selected for the study of which 29 cases found to be positive for malignancy. Two cases found to be false negative. The sensitivity of FNAC for detection of malignancy was 92.59% while specificity 100%. The conclusion is that FNAC can be a very important diagnostic test in detecting malignancy in palpable breast lumps.The research show the final histopathology diagnosis highly correlating with the cytological diagnosis.

Keyword: Cytology, Fnac.

Introduction

As general population becoming more aware about breast lump, people are coming in large number to the clinicians. Any breast lump can be malignant that is why the patients and clinicians are worried. Although histopathology is the most reliable and important diagnostic test for breast lumps, the tests gaining importance are FNAC and Core needle biopsy.[1]

Fine needle aspiration is a rapid, simple, reliable, complication free and cheaper test in diagnosing breast lumps. Surgery can be planned according to fnac report. The second most common malignancy in Indian women is of breast.[2] Local anaesthesis is also not required for this procedure.In 1930 Martin and Lewis first brought this procedure into practice at Memorial Hospital USA.[3]

In the hands of trained and experienced pathologists,the accuracy of FNAC can be equal to that of histopathology.[4] USG or CT guided FNAC or multiple passes in the breast lumps can increase the accuracy of fnac.[5] Most of the breast diseases present with breast lumps. The clinicians mostly follow "triple assessment" protocol. It includes first clinical diagnosis, then mammography and FNAC.

FNAC is very important part of triple assessment. The procedure is expected to be very effective which will avoid further diagnostic biopsy before final definitive surgery.

Materials and Methods

This study was done in the department of Pathology of American International Institute of Medical Sciences, Udaipur between Jan 2016 to July 2017.This is a retrospective study of palpable breast lumps. Slides were collected from histopathology and FNAC records in the respective sections of the department. Request form were used to note down age, sex, and clinical presentation of the patients. Slides of all FNAC and histopathology were reviewed in order to confirm the diagnosis. Findings of FNAC were correlated with histopathology diagnosis. Sensitivity, specificity, accuracy, positive predictive value and negative predictive value were calculated using standard statistical methods. In our cytology section, FNAC for breast lumps is done using 23 gauge needle and 10c.c syringe.

Results

There were 125 cases of FNAC obtained in one and half years period which formed 11% of all FNAC in the department of Pathology. Out of these 125 cases 5 patients were males and rest 120 cases were females. The patients were between the age of 13-79 yrs.

Table 1: Distribution of cases according to age

Age group (yrs.)	Benign and inflammatory		Malignant	
	No. of cases	%	No. of cases	%
0-10	-	0%	-	0%
11-20	8	8.50%	-	0%
21-30	49	52.15%	1	3.26%
31-40	29	30.85%	2	6.45%
41-50	7	7.44%	10	32.25%
51-60	1	1.06%	13	41.93%
61-70	-	0%	3	9.67%
71-80	-	0%	2	6.45%
Total	94	100%	31	100%

Cytodiagnosis Of Salivary Gland Lesions.

Charusheela Rajesh Gore, Pradeep Jadhav, Sarita Jaiswal, Shirish Chandanwale, Punita Kaikal
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Abstract : Objectives: The purpose of the present study was to analyse the relative frequency and distribution of different salivary gland lesions,to evaluate the diagnostic accuracy and efficacy of FNAC in diagnosing these lesions and to correlate the FNAC finding with histopathological diagnosis wherever possible. Method : During the five year period of study ,hundred cases were studied .FNAC was done in eightyone cases and sixtynine cases were available for histopathological examination.Cyological and histological correlation was possible in fifty cases.For cytological examination smears were stained with Leishmann,H & E and PAP. Result : The results were analysed statistically by sensitivity,specificity ,positive and negative predictive values.In our study there was equal distribution of salivary gland lesion among both sexes.Maximum number of lesions were found in parotid followed by submandibular gland.The overall diagnostic accuracy of FNAC in diagnosing the malignant lesions was 90%. Conclusion: The diagnostic accuracy and low false positive and false negative diagnosis obtained in this study warrants FNAC to be utilized as first line diagnostic procedure in the evaluation of patients with suspected salivary gland lesions. Histopathological diagnosis however,still remains the gold standard. [Gore C R et al NJIRM 2013; 4(2) : 134-139]

Key Words: Cytology ,Neoplastic ,Nonneoplastic , Salivary gland

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Introduction: Salivary gland tumours are relatively uncommon neoplasms,representing 2%-6.5% of all head and neck neoplasms. Though uncommon, their spectrum is quiet varied and challenging. A nodule or diffuse enlargement of salivary gland may be caused by a cystic lesion,an inflammation, a degenerative process or a benign or malignant neoplasm. For adequate management ,the exact nature of process must be revealed which can be done by microscopic evaluation[1].

The salivary gland consist of major and minor salivary glands. The parotid ,the submandibular and sublingual gland are major salivary glands.The minor salivary gland are found essentially anywhere in the upper aerodigestive tract including trachea and paranasal sinuses.

Literature search had revealed that almost about 80% lesions occur in major salivary glands.Parotid constitutes the major bulk of about 70% lesions followed by 7-11% in submandibular and < 1% lesions in sublingual gland. Approximately 9-23% lesions occur in minor salivary glands[2].

Clinically ,nonneoplastic lesions of salivary glands can simulate a neoplastic lesion and vice-versa. The accuracy of the clinical examination in diagnosing and differentiating salivary gland lesions is unsatisfactory[3] . Other diagnostic methods like sialography,ultrasonography,CT scan do provide some diagnostic information but are unsuccessful in differentiating the nature and type of various salivary gland lesion[3] . Fine Needle Aspiration Cytology(FNAC) is becoming widely recognized as a practical and useful technique in the diagnosis of salivary gland lesions as it can diagnose these lesions with high accuracy[4].

The purpose of the present study was to analyse the relative frequency and distribution of different salivary gland lesions.To evaluate the diagnostic accuracy and efficacy of FNAC in diagnosing these lesions.A correlation has been made between original specific cytological and final histopathological diagnosis wherever surgical biopsy has been done.

Material and Methods : The work represents the retrospective and prospective study of salivary gland lesions carried out in Pad. Dr. D.Y. Patil Medical College,Pimpri,Pune. Prior to the study ethical committee clearance was obtained. A total of hundred cases were studied during a period of five years from August 2007 to June 2011. FNAC was done in eighty one cases.

After obtaining the consent from patients or relatives,complete clinical history taking and physical examination were performed.The relevant

Dr pradeep Jadhav

Research papers published

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National Journal of Integrated Research in Medicine (NJIRM) 2013;4(2):134-139

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2.Accuracy of Fine Needle Aspiration in Categorization of Thyroid Lesions

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3.Accuracy of Fine Needle Aspiration Cytology in Categorization of Breast Lumps.

Journal of Pharmaceutical and Biological Sciences (JPBS) 2017 ;5(6)

Pradeep Jadhav ,Megha Pandey ,Ashish Pandey

4 Histopathological patterns of Abnormal Uterine Bleeding at a Tertiary Hospital

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Pradhan M.Pagaro, Pradeep Jadhav.

Evaluation of histopathological cases of intestinal obstruction and perforation: A retrospective study of tertiary care centre

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Abstract

Background: Patients with intestinal perforation and obstruction offer a great work load for surgery. Difference is present between developed and developing countries. Histopathology can provide a definitive evidence of the etiology. **Aims and Objectives:** To study the various etiologies of intestinal perforation and obstruction by histopathological examination. **Materials and Method:** One hundred and thirty nine patients were studied in the Department of Pathology, People's Medical College and research center, Bhopal, from 2015 Jan to 2018 Dec. Gross examination and histopathology was performed on all the intestinal resected specimens. **Results:** Mean age of study cohort was 39.30±18.41 years with male preponderance (68.3%). Prevalence of obstruction was 61.87% and perforation was 38.12%. Histopathology revealed non-specific inflammation in 30.9%, adenocarcinoma in 15.10%, chronic granulomatous lesions in 16.54%, ischemic bowel in 3.59%, meckel's diverticulum in 3.6% and foreign body reaction in 2.9%. Etiological factors found in intestinal obstruction was adenocarcinoma (23.25%) followed by non Specific inflammation (18.60%) and chronic granulomatous lesion (16.27%). Etiological factors found in intestinal perforation was non-specific inflammation (50.94%) followed by chronic granulomatous lesion (16.98%) and Ischemic Bowel Disease (3.77%). **Conclusion:** Obstruction was more prevalent compared to intestinal perforation. Among obstruction adenocarcinoma, non Specific inflammation and chronic granulomatous lesion were more common whereas in perforation non-specific inflammation, chronic granulomatous lesion and Ischemic Bowel Disease were more common.

Key Words: adenocarcinoma, perforation, obstruction, intestinal resection.

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INTRODUCTION

In patients with compromised small and large intestine features of acute intestinal obstruction or perforation can be noticed because of wide range of pathologies. Intestinal resection is used in case of failure of conservative management.[1]

Previous studies have reported a wide range of causes of acute intestinal obstruction and this difference is based on the demography.[2] In developing country like India, tuberculosis accounts for more than half of the cases of small bowel obstruction. However, large bowel obstruction remains the matter of further research due to the presence of varied number of causes such as tumours, diverticulitis, volvulus or fecal impaction. Intestinal perforation remains one of the serious factors associated with high morbidity and mortality. Causes of intestinal perforation varied based on the demography of the study.[3,4] Most common causes of intestinal perforation include typhoid, tuberculosis, HIV infection and amoebiasis. Several non infectious factors including

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gender wise distribution of cases presenting with cervical lymphadenopathy: A retrospective study

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Abstract

Introduction: Cervical lymphadenopathy is a common clinical presentation of various diseases its FNAC serves as an excellent clue to the underlying disease. The presentation of cervical lymphadenopathy varies in different age groups and in both the both the gender as well.

Objective: To analyze the spectrum of lesions causing cervical lymphadenopathy as well as to see the age and gender wise distribution of cases of tubercular lymphadenitis.

Materials and Methods: It is a retrospective study and included three years data. A total of 222 cases that underwent FNA for cervical lymphadenopathy were recorded with their clinical history. For all cases, MGG, Pap and AFB slides were re evaluated and results were recorded. Epidemiological variables were analyzed by cross tabulation to assess their relationship.

Result: Out of 222 cases 117 cases were of Tubercular lymphadenitis followed by 81 cases of reactive lymphadenitis. 10 cases presented as metastatic deposit of squamous cell carcinoma, 9 with Lymphoma (NHL) followed by 5 cases of adenocarcinoma deposits. Females to males ratio in Tuberculosis lesions was 1.3:1

Conclusion: In both pediatric and adult population Tuberculosis was an important cause of cervical lymphadenopathy while in children less than two years reactive lymphadenitis was more common. Tuberculosis was more common in females as compared to males and here FNAC is an inexpensive and valuable fist line tool in assessing cervical lymphadenopathies.

Keywords: Cervical lymphadenopathy, Tuberculosis, FNAC, Age and gender.

Introduction

Cervical lymphadenopathy is a common clinical presentation; the presentation could be either as an isolated lymphnode or as a part of generalized lymphadenopathy. An abnormal increase in size, number and consisitency is known as lymphadenopathy.[1] lymphadenopathy is the clinical manifestation of local or systemic diseases and its FNA serves as an excellent clue to the underlying disease. For extra pulmonary tuberculosis lymphadenopathy is the most common clinical presentation. There are approximately 600 lymphnodes in the human body which are submandibular, axillary or inguinal lymphnodes may normally be palpable in healthy people. Amongst all the lymphnodes cervical lymph nodes are the most common site of involvement and are reported in 60-90% cases. Cervical Lymphadenitis is noted in a wide spectrum of diseases ranging from infections, in tumors like Hodgkins and Non Hodgkins lymphoma, autoimmune diseases (SLE, rheumatoid arthritis) & in metastatic deposits of squamous cell & adenocarcinoma etc. Based on the duration cervical lymphadenopathy can be divided into acute, subacute and chronic lymphadenopathy. Acute lymphadenopathy is lymphadenopathy which persisits for two weeks duration, subacute lymphadenopathy has two to six weeks duration and chronic lymphadenopathy is defined as any lymphadenopathy which doest resolve by six weeks.[2]

FNAC is now emerging as safe, rapid, simple, inexpensive and reliable method for diagnosing the enlarged lymphnodes with high degree of efficacy and thus help in establishing the diagnosis of various masses and lesions at various sites and organs.[3-7] FNA is now considered as a valuable diagnostic tool and provides an ease in the follow up of the patients presenting with malignancy and identification of recurrence or metastasis.

In lymphnodes malignancies are commonly metastatic in nature however lymphomas range from 2 to 15%[8] and for their diagnosis the gold standard is the histopathological examination. In the developing countries like India amongst the inflammatory lesions tuberculosis is one of the most common infective causes of superficial lymphadenopathy.[9,10] This article reviews the evaluation of patients with lymphadenopathy, evaluating the cause of it in different ages with the help of FNAC and to evaluate the variation in the presentation of cervical lymphadenopthy in both the genders.

Aims and Objectives

1. To analyze the cytological spectrum of lesions causing cervical lymphadenopathy.
2. To see the Age and Gender wise distribution of cases of tubercular lymphadenitis.

Materials and Methods

It is a retrospective study carried out in the Department of Pathology at a Tertiary care Centre. The study was conducted to evaluate the various cytomorphological features of inflammatory, neoplastic and non-neoplastic lesions of lymphnodes by FNAC; in patients presenting with

RESEARCH PAPER

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F FINE NEEDLE ASPIRATION CYTOLOGY IN EVALUATION OF BREAST ES IN PREGNANT AND LACTATING WOMEN: AN EXPERIENCE WITH 32 CASES.

ology

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ABSTRACT

Introduction: Evaluation of breast masses during pregnancy and lactation is challenging. FNA of these lesions and cytological interpretation is considered problematic due to atypical inherent to secretory change in glandular epithelia.

Objective: To evaluate the role of fine needle aspiration cytology in the diagnosis of palpable breast masses during pregnancy and lactation.

Methods: The present study was conducted at the department of pathology, People's medical college and research centre, Bhopal. Cases of breast mass in pregnant or lactating women presenting to OPD for FNA over a period of (January 2014–June 2017), were retrospectively evaluated.

Results: Around 32 cases of breast mass in pregnant and lactating women are included in the study . Present study focuses on most common differential diagnoses: galactocele (2 cases), fibroadenoma (7 cases), lactating adenoma (9 cases), mastitis and abscess (6 cases), Masses with Benign findings (4 cases), Fibrocystic disease (1 case), suspicious for malignancy (1 case) and PABC (2 cases)

Conclusion: Breast lesions detected during pregnancy or lactation are difficult to diagnose due to the hormone-induced physiological changes occurring in breast. However, FNA play an important role in evaluating breast masses in pregnant and lactating women.

KEYWORDS

Lactating adenoma, Fine needle aspiration (FNA), pregnancy-associated breast cancer (PABC)

Introduction

Physiological changes occur in breast during pregnancy and lactation. Due to elevated circulating hormones there is added ductal and lobular growth, increased vascularity and a decrease in stroma which results in increased breast density & makes breast evaluation challenging in these patients. Radiological assessment for pregnant and lactating women under age of 30 years, ultrasound is the imaging test of choice given the lack of radiation exposure[1].

Fine needle aspiration (FNA) of breast masses in pregnant or lactating women & cytological analysis is considered problematic due to atypia innate to secretory change in glandular epithelia[2].

Breast adenomas are defined as circumscribed neoplasm composed of proliferations of cytologically bland epithelial structures with inconspicuous stroma[7].

A pregnancy-related breast disorder is defined as a diagnosis made during pregnancy, within one year of post-partum or during lactation. Because of the variability in the length of lactation, cancer occurring up to 1 year after delivery has been accepted as the standard definition in most recent series[3].

Most disorders are analogous to those in non-pregnant women, though there are a number of conditions distinctive to pregnancy and lactating women. These conditions almost always present as a palpable mass and are often a source of great anxiety for the woman and her family[4].

The main differential diagnoses for palpable breast masses in pregnant or lactating women include: galactocele, fibroadenoma, lactating adenoma, mastitis and abscess, benign findings consistent with pregnancy or lactational changes, fibrocystic disease with lactational changes and Pregnancy associated Breast cancer (PABC)[1].

AIMS AND OBJECTIVES

1. To evaluate the role of Fine needle aspiration cytology in diagnosis of palpable breast masses in pregnant and lactating women.
2. To delineate the cytomorphologic features seen in cancer of the breast during pregnancy and lactation and to compare them to the cytomorphologic parameters in benign conditions.

MATERIAL AND METHODS

Present study was carried out at department of Pathology, People's medical college and research centre, Bhopal. Cases of breast masses in pregnant or lactating women presenting to OPD for FNA over a period of (January 2014–November 2017), were retrospectively evaluated. FNAC smears along with relevant clinical information related to age, sex, clinical diagnosis, geastational age, surgical information, radiological findings and findings on follow up of cases were retrieved from cytopathology records. The findings of FNA were reviewed, analyzed, tabulated and correlated with the histopathologic diagnosis of the cases in which breast biopsies were performed.

Results

Total of 32 cases with palpable breast masses in pregnant and lactating women between 18-38 yr of age (Table-1) presented to OPD for FNA during the study period. Size of mass ranged from 1.0 to 4.5 cm. The estimated gestational age at the time of FNA ranged from 16- 32 week, postpartum or lactating as shown in Table 2. The follow up was done regularly for a period of 1-1.5 yr and in few cases mass(es) regressed after delivery or discontinuation of breast feeding and the cases in which mass(es) persisted repeat aspirations was done with follow up or were operated and tissue was sent for histopathological examination.

Table 1 Distribution of cases according to Age

Age Group	No. of cases
15-20 yrs	03
21-25 yrs	15
26-30 yrs	10
31-35 yrs	03
36-40 yrs	01
Total	32

Table 2 Distribution of cases according to gestational age

Gestational Age	No of cases
No. of breast masses that arose during pregnancy (16-32 weeks)	05
No. of breast masses that arose during post partum period or lactation	27

Reproducibility of "The Bethesda System for Reporting Thyroid Cytopathology": A Retrospective Analysis of 107 Patients

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Abstract

Objectives: Fine-needle aspiration cytology (FNAC) has emerged as an indispensable tool to discriminate thyroid lesions into benign or malignant for appropriate management. The need for simplicity of communication and standardization of terminology for thyroid FNAC reporting led to introduction of "The Bethesda system for reporting Thyroid Cytopathology" (TBSRTC) in a conference held at the National Cancer Institute in 2007. This study aims at establishing the reproducibility of TBSRTC for diagnosing thyroid lesions. **Materials and Methods:** The present study comprised thyroid FNAC from 107 patients retrospectively over a period of 1.5 year (June 2013 to December 2014), which were reviewed by two trained cytopathologists and re-categorized according to TBSRTC. The interobserver variation and reproducibility of the reporting system was statistically assessed using Cohen's kappa. **Results:** The cytopathologists were in agreement in 98 out of 107 cases (91.5%). Maximum concordance was noted in benign category (91 of 96 cases; 92.85%), followed by 2 cases each in nondiagnostic/unsatisfactory (ND/US) and follicular neoplasm/suspicious for follicular neoplasm (FN/SFN) category (2.04% each) and 1 case each in atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), suspicious for malignancy (SUS), and malignant category (1.02% each). The highest diagnostic disagreement was noted among ND/US and benign and FN/SFN categories. **Conclusion:** The utilization of TBSRTC for reporting thyroid cytology should be promoted in our country because it provides a homogeneous, standardized, and unanimous terminology for cytological diagnosis of thyroid lesions. The present study could substantiate the diagnostic reproducibility of this system.

Keywords: Bethesda System, fine-needle aspiration cytology, reproducibility, thyroid

Introduction

Thyroid diseases are frequently encountered in India and approximately 42 million people in India suffer from thyroid diseases.[1] The prevalence of a palpable thyroid nodule in India is approximately 12.2%, however the incidence of thyroid cancer is 8.7 cases per 100,000 population per year.[2] As a palpable thyroid nodule may not always be malignant, it is important for the clinician to distinguish between benign and malignant nodules for appropriate management. Consequently, fine needle aspiration cytology (FNAC) surfaced as an indispensable first-line diagnostic tool to classify palpable thyroid nodules into benign or malignant, thereby reducing unnecessary surgeries in benign thyroid nodules.[3]

In the pre-FNAC era, only 14% of surgically resected thyroid nodules were malignant. However, with the introduction of FNAC, the surgical resection of malignancy increased up to 50%.[4] Traditionally, the use of diverse nomenclature and diagnostic criteria by pathologists for reporting of thyroid nodules has limited the understanding of cytopathology reports by clinicians, thus hindering a definitive management.[5]

In 2007, The Bethesda system for reporting thyroid cytology (TBSRTC) was proposed at the National Cancer Institute Thyroid FNA State of Science Conference at Bethesda, Maryland. This reporting system includes suggestions regarding the layout of the report, adequacy of the sample, diagnostic category, risk of malignancy, and proposed

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Original Article

Clinicopathological Diagnosis of Leprosy: Comparative Evaluation of Three Staining Methods for Acid Fast bacilli in Slit Skin Smears and Biopsy Specimens

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Detection of acid fast bacilli (AFB) in slit smears and histopathological specimens is of paramount importance for diagnosis and classification of leprosy. In this study the results of conventional staining technique for AFB has been compared with modified rapid AFB and Fite Faraco stain in slit skin smears and punch biopsy specimens from clinically diagnosed cases of leprosy. Processed skin biopsies and slit skin smears of 42 patients attending outdoor clinic of a tertiary care centre were stained with three stains viz Fite Faraco, modified Rapid AFB and conventional Ziehl Neelsen staining. According to clinical diagnosis the maximum number of patients belonged to Borderline Tuberculoid leprosy which correlated with histopathological diagnosis of skin biopsy. Clinical and histopathological correlation was not observed in 14/42 cases - histopathological categorization of biopsies from these cases revealed Indeterminate leprosy (9 cases), Tuberculoid Leprosy (2 cases), Borderline Lepromatous (1 case), Histoid Leprosy (1 case) and Mid- Borderline Leprosy (1 case). Maximum positivity for AFB was seen with Fite Faraco staining followed by modified Rapid AFB both in the biopsy specimens and slit skin smears. Fite Faraco staining showed highest sensitivity in both paucibacillary and multibacillary cases followed by modified rapid AFB and conventional AFB staining. Though biopsy and slit skin smears have their individual diagnostic advantages and limitations, biopsy deserves to be viewed as gold standard in case of difficulty in arriving at a confirmed diagnosis. Findings of this study need to be validated in a larger number of leprosy cases at community level studies and correlated with classification currently recommended by WHO and NLEP.

Keywords : Granuloma, Slit Skin Smears, Acid Fast Bacilli, Leprosy, Fite Faraco, Modified AFB staining

Introduction

Despite the efforts of the National Leprosy Eradication Programme (NLEP) strategies and plans the fact remains that India continues to account for 60% of new cases reported globally each year and is among the 22 "global priority countries" that contribute 95% of world numbers of leprosy warranting a sustained effort to bring the numbers down (Lockwood 2002, Gurung et al 2019, Srinivas et al 2002). Clinically, leprosy is

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Original Research Article

Ki-67 expression in the lesions of uterine cervix

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Abstract

Background: Malignant transformation and uncontrolled cellular proliferation are the key factors in the development of cervical cancers. The aim of this study was to assess the proliferative activity by using Ki-67 proliferative marker. **Methods:** We used immunohistochemical methods to study the expression of Ki-67 in the specimens of 14 Patients of cervicitis, 6 patients of LSIL, 6 Patients of HSIL and 14 patients of squamous cell carcinoma. **Results:** Ki-67 showed positivity in lower one third of epithelium in all the 14 patients of non specific cervicitis, positivity in lower two third of epithelium in all 6 patients of LSIL and 6 patients of HSIL. Also, Ki-67 was positive in entire thickness in all cervical carcinoma patients. This observation points that Ki-67 being the index of cell growth fraction is found to increase with increase in cell proliferation which in turn indicates the degree of malignancy. **Conclusion:** This study showed Ki-67 is positively associated with increase in grades of cervical lesions and carcinoma cervix with statistically significant p value of 0.001. Ki-67 antigen could be an important factor to identify women at higher risk for progression to cervical cancer.

Key Word: Ki-67 Antigen; Immunohistochemistry, HSIL, LSIL

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INTRODUCTION

Worldwide, cervical carcinoma is the fourth-most common cause of cancer and fourth common cause of death in women.[1] In India, Cervical cancer is a leading cause of cancer related death among women especially those from lower socioeconomic group contributing to about one third of total world population.[2] In India, the peak age of incidence of cervical cancer is 55-59 years.[3] Human papilloma virus is the prime cause for carcinoma cervix. Of these, 16 and 18 types of HPV are categorised under high risk types. In India, HPV prevalence among cervical cancer patients varied from 87.8% to 96.67%. Carcinoma cervix is known to develop from premalignant lesion, intraepithelial neoplasia. It takes nearly 5 to 15 years to progress to invasive carcinoma.[4]Hence it is very essential to identify precancerous lesions for appropriate treatment. No form of malignancy better documents the most remarkable benefits of effective screening, early diagnosis, and curative therapy than does the carcinoma of cervix.[4] Our aim was to evaluate the expression of Ki-67 proteins in cervical biopsies and to assess the utility of Ki-67 in diagnosing and grading cervical lesions. Ki-67 is a marker of cell proliferation, and is expressed in all phases of cell cycle except in G0. It has a function of growth in human tumor, and its expression could suggest the degree of malignancy. The interaction of E6 and E7 oncoproteins of HPV DNA in the host cell disturbs the cell cycle and express themselves by the abnormal expression of proteins, including Ki-67.[5]Its positivity demonstrated by immunohistochemistry indicates the increasing proliferation in low and high grades of intraepithelial lesions.[6]

MATERIALS AND METHODS

Specimen of 40 cervical biopsy were selected for analysis from the archives of the Department of Pathology, Coimbatore Medical College, Coimbatore (Tamil Nadu). They were categorized based on their histomorphological features in haematoxylin and eosin stained sections into non specific cervicitis (n=14), low grade squamous intraepithelial lesion (n=6), high grade squamous intraepithelial lesion (n=6) and invasive squamous cell carcinoma (n=14). The tissue had been fixed in buffered formaldehyde, embedded in paraffin, cut at 4 micron, and

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Oral Squamous Cell Carcinoma In Bundelkhand Region: Epidemiological And Histopathological Profile

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Abstract: Aim: To Study The Epidemiological And Histopathological Profile Of The Oral Cancers In Adult Patients In Bundelkhand Region. Our Aim Is To Describe The Oral Cancers In Adult Patients, Theirs Frequences And Their Histopathological Characteristics. **Material And Method:** The Tissue Material For The Study Was Obtained From Various Out-Patients And In-Patients Admitted In ENT And Surgery Department In MLB Medical College , Jhansi From January 2014 To July 2015.A Total Of 55 Cases Were Collected. **Results:** In Our Study, Patients Whose Age Is Above 40 Years Were The Age Most Affected By The Oral Cancer With 89.1%, While Patients Whose Age Is Less Than 40 Years Old Represented Only 10.9% Of The Cases Affected By The Oral Cavity Cancer. In Our Study, Males Accounted For 69% Of All Cases. Oral Well Differentiated Squamous Cell Carcinoma (OSCC) Was The Most Dominant Histological Type In 80% Of Cases. Tobacco Was The Most Dominant Risk Factor With 70% Of All Cases. **Conclusion:** Our Results Correlate Well With Previously Published Clinicopathological Data On Comparable Studies.

Keywords: Oral Cavity,Squamous Cell Carcinoma(SCC)

Date of Submission: 24-02-2018 Date of acceptance: 12-03-2018

I. Introduction:

Oral Cancers Are Emerging As A Major Public Health Problem In India, Which Are Related To Lifestyle, Have A Lengthy Latent Period And Need Dedicated Infra Structure And Human Resource For Treatment. More Than 95% Of Cancers Of The Oral Cavity Are Squamous Cell Carcinomas. They Constitute A Major Health Problem In Developing Countries, Representing A Leading Cause Of Death .Oral Squamous Cell Carcinoma Is An Aggressive Epithelial Malignancy That Is The Sixth Most Common Neoplasm In The World Today. There Are 500000 New Cases A Year Worldwide Of This Two Thirds Occur In Industrialized Nations.. It Is The Sixth Common Cause Of Death In Males And Seventh In Females. Oral Squamous Cell Carcinoma Usually Develops In Male In The 4th And 6th Decade Of Life. In Recent Days Despite Of Advantage Of Treatment, The Overall Long Term Survival Has Remained At Less Than 50% For The Past 50 Year[American Cancer Society]. **[Cancer Facts And Figures 2007].**

The Cancer And Its Treatment Involve Pain And Disfigurement And It Can Affect Speech, Swallowing And Breathing ***[Chaturvedi, P. Head And J Can Res Ther 2009]***. Clinicopathological Criteria Are Very Important For The Management And Prediction Of Outcome Of Patients. However, It Has Been Observed That Within A Group Of Patients Sharing The Same Clinicopathological Features, There Is Still Significant Variation In Its Outcome. Studies On The Carcinogenesis In Oral SCC Can Lead To Understand Its Biological Behaviour And To Reveal The Underlying Mechanisms Of Cancer Progression And Therapy Resistance [***Lingen W. Head And Neck, 2010***.] Clinical, Epidemiological And Laboratory Data Has Established That Tobacco Usage And Alcohol Consumption As The Major Source Of Oral Cancers [***Chin D, Boyle GM, Theile DR, Parsons PG, Coman WB,2004***]

Smoking Habits That Increase The Risk Of Developing Oral SCC Are Smoking Black Tobacco (Compared To Blond Tobacco), Smoking At A Young Age, Long Duration, High Number Of Cigarettes Per Day, And Deep Smoke Inhalation [***Thomas GR, Nadiminti H, Regalado J. 2005***]. Avoiding Cigarettes And Alcohol Could Prevent About 90% Of Oral Sccs. Tobacco Chewing Is A Major Cause Of Oral And Oropharyngeal SCC In The Indian Subcontinent, Parts Of South-East Asia, China And Taiwan, Especially When Consumed In Betel Quids Containing Areca Nut (***Znaor Et Al., 2003***)[***Brugere J, Guenel P, Leclerc A, Rodriguez J, 1986***]. In India, Chewing Accounts For Nearly 50% Of Oral And Oropharyngeal Tumors In Men And Over 90% In Women (***Barnes Et Al., 2005***) [***Benhamou CA, Laraqui N, Touhami M, Chekkoury A, Benchakroun Y, Samlali R, Kahlain A, 1992***].

Original Article

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Immunohistochemical Expression of p16INK4A in The Lesions of Uterine Cervix

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ABSTRACT

Background: Cervical cancer is the major cause of cancer deaths among women. Globally, around 5,70,000 new cases of cervical cancer and 3,11,000 deaths occurred in the year 2018. In India, Cervical cancer is a leading cause of cancer related mortality among women and the number of deaths is 60,000 per year among 97,000 diagnosed patients, especially those from lower socioeconomic group. Human Papilloma Virus (HPV) plays a crucial role in causing cervical dysplasia. This is done by upregulating p16INK4A, a cyclin dependent kinase inhibitor through interaction with cellular regulatory proteins. Hence p16INK4A can be used as a biomarker, since it is directly related variable for the presence of HPV. This study was conducted to evaluate the expression of p16INK4A in benign, premalignant and malignant cervical lesions and to assess its utility in diagnosing and grading cervical lesions.

Methods: A total of 80 cervical specimens categorized histopathologically into nonspecific cervicitis, low grade squamous intraepithelial neoplasia (LSIL), high grade squamous intraepithelial neoplasia (HSIL) and squamous cell carcinoma cervix were included in this prospective study of one-year duration. Immunohistochemical study of p16INK4A was interpreted qualitatively and semi-quantitatively by Allred scoring system (0 to 8 points) which measures the proportion of stained cells and intensity of staining of cells. The collected data were statistically analyzed by ANOVA and chi square test.

Result: Qualitative method showed absence of p16INK4A expression in all nonspecific cervicitis, 16.7% (2/12) LSIL, 100% (12/12) HSIL and 100% (28/28) squamous cell carcinoma cases showed p16INK4A positivity. Allred scoring of p16INK4A showed 66% (8/12) HSIL and 85.7% (24/28) squamous cell carcinoma cases with score 7 positivity. Hence high grade lesions showed higher expression of this marker.

Conclusion: IHC expression of p16INK4A showed increasing degree of expression from benign to premalignant and malignant lesions suggesting its diagnostic and prognostic value in the cervical cancer management.

Keywords: p16INK4A, Intraepithelial Neoplasia, Cervix Carcinoma

Introduction

Worldwide, cervical carcinoma is the fourth-most common cause of cancer and fourth common cause of death in women.[1] Every year, cervical cancer is diagnosed in about 5,00,000 women globally and is responsible for more than 2,80,000 deaths annually.[2] In India, Cervical cancer is a leading cause of cancer related death among women, especially those from lower socioeconomic group contributing to about one third of total world population.[3] Human papilloma virus is the prime cause for carcinoma cervix and its prevalence among cervical cancer patients varied from 87.8% to 96.67%.[4] Molecular studies have shown that HPV 16 and 18 are most common oncogenic types involved in causing carcinoma cervix. HPV infection of cervix is sexually transmitted. Young and sexually active women are at highest risk for HPV infection and preinvasive cervical neoplasia.

Carcinoma cervix is known to develop from premalignant lesion, intraepithelial neoplasia. It takes nearly 5 to 15 years to progress to invasive carcinoma.[5] Hence it is very essential to identify precancerous lesion for appropriate treatment. No form of malignancy better documents the most remarkable benefits of effective screening, early diagnosis, and curative therapy than does the carcinoma of cervix.[6] However detection and interpretation of HPV infection in cervical specimens are difficult. Hence indirectly assessing the presence of HPV infection by analyzing a step-in pathogenesis of HPV cell cycle is of utmost importance.

Various studies have highlighted the role of p16INK4A as an excellent marker of cervical carcinoma. Its expression is associated with the progression of disease. It is directly related to the presence of HPV.[7] p16INK4A belongs to cyclin-dependent kinase Cdk4 6 inhibitors. It is encoded by tumor suppressor gene INK 4A. It has an important role in the regulatory pathway Cdk-Rb-E2F. The protein product of the gene, p16INK4A, prevents pRb phosphorylation by inactivating cyclin dependent kinase Cdk4 6. pRb keeps binding E2F transcription factors and rests the cell in G1 phase and does not pass DNA for replication.[8] In

Dr. Prdeep S. Jadhav

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Research Article

To Study the Histopathological Forms of Thyroid Lesions Observed in Tertiary Care Hospital

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Scopus

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ABSTRACT

Introduction: The thyroid organ is a butterfly-model organ made out of globular right and left flaps associated in the midline by a meagre organisation named isthmus. Disorder related to thyroid consists of a group of disease of endocrines. All of these disorders may be associated with clinical conditions of hyper as well as hypo thyroids. Surgical excision and pathological evaluation are vital to establish a proper diagnosis.

Aim: The study to show the histopathological forms of thyroid lesions observed in a tertiary care hospital.

Materials and Methods: The present cross-sectional study was conducted for 2 years. Inclusion criteria: The test population included patients with thyroid pathology in a specified period. Thyroidectomy specimens including lobectomy, partial thyroidectomy, subtotal thyroidectomy and total thyroidectomy. Exclusion criteria: Patients with other disorder than thyroid were excluded.

Results: The total number of studied cases was 225 cases (table 1). The reviewed cases were classically categorized into two main groups; Non- neoplastic (132; 58.5%) and Neoplastic (93; 41.5%). Papillary carcinoma was the most frequent thyroid cancer accounting for most of the thyroid cancers.

Conclusion: Thus, in conclusion, females accounted for a higher number of patients with neoplastic thyroid lesions and the prevalence peaked at a younger age. Present study finding suggests that papillary carcinoma appears much before development so diagnosis should be fastened.

Key Words: Histopathological, Thyroid, Papillary carcinoma, Neoplastic thyroid lesions

INTRODUCTION

The thyroid organ is a butterfly-model organ made out of globular right and left flaps associated in the midline by a meagre organisation named isthmus [1,2] Disorder related to thyroid consists of a group of commonly come across the disease of endocrines. [3-5] The thyroid gland is a primary endocrine organ to form during foetal growth. Its development starts at four weeks of conception, it develops at around a month of conception starting from foregut endoderm near the baseline of the tongue, it extends gradually at the fifth month when the foetus develops.[6] Thyroid partakes widespread lymphatic drainage comprising numerous points of lymph nodes, which are not inadequate to the pre-laryngeal (or Delphian), pre- and paratracheal, retropharyngeal, retroesophageal, and internal jugular lymph nodes. The process becomes very important in the classification and staging of thyroid carcinoma. At that time careful lymph node and the dissection of it may be necessary for the examination for metastases.[4,7] The occurrence of goitre in regions of unembellished iodine deficiency can be as in elevation as 80%. Inhabitants at specific threat tend to be out-of-the-way and live in hilly areas in South-East of Asia, Latin America and in Central of Africa.[4,8] Between 2006-2008 and 2012-2014, the ASRs for Thyroid cancer in India increased from 2.5 to 3.5/100,000 women (+37%) and from 1.0 to 1.3/100,000 men (+27%).[9] Thyroid disorders are four times more in females than in males[10,11] The endocrine systems glands may exaggerate by a diversity of illnesses and sickness which ranges from useful, functional and immunological intermediated which leads to growth and formation to neoplastic lesions.[12]

The greater part of thyroid lesions consists of non-neoplastic lesions and 5% of the thyroid lesions are neoplastic whereas remaining of the lesions are due to inflammatory or developmental reasons.[13] Multinodular goitre is the communal

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Pathology Section

Alternative Rapid Methods for Coverslip Removal: A Comparative Study

MAHESH H KARIGOUDAR[1], SACHIN SHIVAJI KAPSE[2], DISHA B SHANKAR[3], SHIKHA SHARMA[4], DIVYA PRAFULLA YERRAGUNTLA[5]

ABSTRACT

Introduction: In 21st century, the focus is to develop method which yields outcome in shorter tenure, and are rapid, safe and obtain quicker results. In this study, non-traditional methods were used for removal of coverslip and was compared with traditional methods.

Aim: To compare the time taken for coverslip removal of old faded slides with five different methods.

Materials and Methods: Faded slides of one year to more than 10-year-old were included in the study. Total of 90 slides were subjected to five different methods of coverslip removal like xylene at room temperature, xylene at 56° Celsius (C), Petrol, Diesel and Freezing method. Time taken for each method was noted.

Results: The mean time taken for removal of coverslip was least with freezing method followed by xylene at 56°C, Petrol, xylene at room temperature and diesel.

Conclusion: Alternative methods are safe, rapid and has less turn around time compared to routine use of xylene. Hence, can be successfully used for removal of coverslip.

Keywords: Freezing, Petrol, Restaining, Xylene

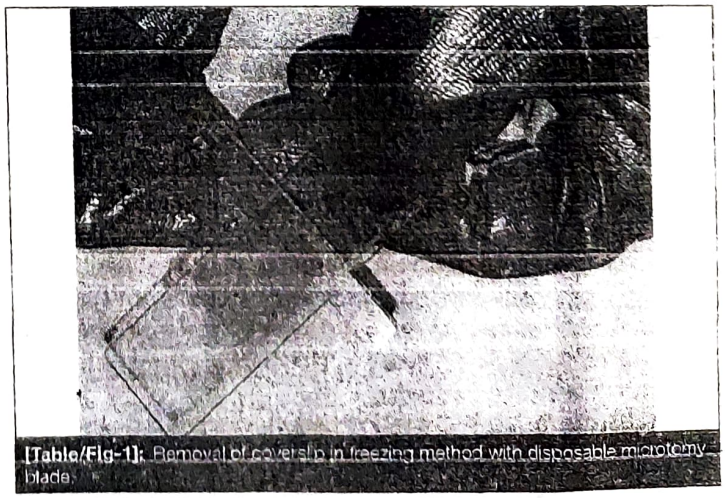
INTRODUCTION

Rapid restaining of archival slides is impossible without decreasing the time spent on coverslip removal [1-3]. Most often, old H&E stained slides show fading of stains over the period of time, unable to view for diagnosis or review. The archival slides may contain diagnostic sections of rare, extinct, interesting or classical cases which might not be easily available nowadays. Old slides can also be used in research, project, dissertation study, IHC, special staining or molecular study [4-6]. In our institute, we undertook the process of restaining of faded slide from the collection boxes meant for postgraduate study material which comprised of histopathology, haematology and cytology slides. Slides were ranging from one year to 22-year-old. The slides were divided into 40 slides/batch and were subjected to restaining. It took eight months for restaining 1200 slides and more than one third time was spent for removal of coverslip. Average time taken for restaining procedure is 100-120 hours which includes 36 to 48 hours for removal of coverslip. There is need of the hour to cut down the turn around time for coverslip removal. Hence, the aim of this study was to use alternate methods for coverslip removal which were safe, reliable and with available resources and faster as compared to routine xylene method.

MATERIALS AND METHODS

Total 90 archival slides were chosen for the prospective study which was conducted in Department of Pathology for the duration of eight months. Distyrene, Plasticizer and Xylene (DPX) were the mounting media in all slides. They were grouped according to duration into three major group as 1 to <5-year-old, 5 to <10-year and >/=10-year-old. According to coverslip size, they were divided into three subgroups; one pair of slide was taken with coverslip size 22x22 mm, 22x30 mm and 22x40 mm. In total six slides were included in each of these subgroups and total 18 slides under each major group. Five methods used for removal of coverslip were; Freezing, Xylene at room temperature, Xylene at 56°C, Petrol and Diesel. The chemicals were taken into glass beaker which were labeled and had proper lid for closure. The slides were labelled with diamond pencil and a unique number was allotted to each slide. First alphabet designated method used, middle number represented duration of slide and last number represented size of coverslip. Number 0, 1, and 2 were designated for slide duration 1 to <5-year-old, 5 to <10-year and >/=10-year-old, respectively. Similarly, number 0, 1, and 2 were given for coverslip size 22×22 mm, 22×30 mm and 22×40 mm. For example, F00 slide number means Freezing method was used, slide is 1 to <5-year-old and coverslip size is 22×22 mm.

In freezing method, the slides were kept for 10 minutes in freezing chamber (Temperature 0° to -4°C) of domestic refrigerator with coverslip facing downwards. Then after every five minutes disposable microtomy blade was passed in between the slide and coverslip to check whether coverslip was popping up easily or not. Time was noted from slide kept in freezer compartment and coverslip popped out easily. To avoid cut related injury cut resistant gloves were worn [Table/Fig-1]. For xylene at 56°C, water bath was used. The slides were kept in a glass jar and immersed inside the water bath maintained at a temperature of 56°C. Time was noted immediately after inserting jar containing slides in water bath till coverslip fell off from slides. For petrol and diesel, plastic airtight jar with cap was used. The time was noted immediately after immersing slide in chemical until coverslip detached out themselves. After the coverslips got separated, the slides were taken for restaining. Broken slides, slides with broken coverslip and slides with tissue loss during processing were excluded from study.



[Table/Fig-1]: Removal of coverslip in freezing method with disposable microtomy blade

RESULTS

In conventional xylene method (Room temperature), the mean time for removal of coverslip was 52 hour 10 minutes. Mean duration in which

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Haematological Profile of Patients with Various Types of Malaria

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ABSTRACT

Background: Malaria is a disease that is known to be a life-threatening infectious disease and is associated with calamitous complications in many cases and can have drastic and far-reaching consequences in a population. It is one of the parasitic infectious diseases that may affect haematological parameters. Most common haematological complications associated with malaria are Anemia, leukocytosis, thrombocytopenia and leucopenia. The magnitudes of these changes vary with endemic malaria, background hemoglobinopathy, nutritional status, demographic factors and immunity from malaria.

Aim: The main objective of this study is to evaluate the haematological profile of patients infected with malaria.

Material and methods: Total 120 malarial positive patients with the cases of haematological disorders were included in this study. From the patient's Blood samples referred for peripheral blood, the smear was collected in ethylenediaminetetraacetic acid (EDTA) and were also analyzed for malarial parasites with conventional microscopy. Giemsa stain was used for Peripheral smear and also PCV, Hb and WBCs Total counts, RBC morphology, and WBCs differential count and platelet counts are also done.

Result: In this study, the main clinical feature was chill fever. Anaemia was seen in almost all cases of malaria. Leucopenia was seen in 29.17% of cases. The incidence of leucopenia was 29.4% in *P. vivax* and in *P. falciparum* was 27.6% and 33.3% in both *P. vivax* and *falciparum*. Monocytosis was seen in 48.24% cases of *P. vivax* and 17.24% cases of *P. falciparum* and 33.33% of incidence in both *P. vivax* and *falciparum*. The significant co-relation between thrombocytopenia and Malaria was found in this study.

Conclusion: This study showed that almost all blood components and is a true haematological infectious disease and in *P. falciparum* substantially higher compared with *P. vivax*.

Key Words: Malaria, *P. falciparum*, *P. vivax*, Thrombocytopenia

INTRODUCTION

Malaria is a disease which is considered as life-threatening infectious disease and in several cases, it is associated with calamitous complications and can inflict drastic and far-reaching consequences within a community.[1] About 250 million cases in a year were estimated and between one to two million deaths. [2] This disease is caused by parasitic infection of genus Plasmodium, which gets injected into the human bloodstream through the bite of a female Anopheles mosquito[3]. There are four species of Plasmodium have been known to cause infections in humans such as *P. falciparum, P. vivax, P. ovale, and P. malariae.* However, *P. knowlesi* as another species was known to causes infection in macaque monkeys and reported to cause malaria in humans.[4,5] World Health Organization (WHO) showed about 40% of the world's people are at risk of malaria. About 300 - 500 million cases of incidence with malaria per year and two million deaths per annum globally have been reported by studies.[6] The magnitude of these changes varies with endemic malaria, background hemoglobinopathy, nutritional status, demographic factors and immunity from malaria[7,8,9] Parasitic infection such as malaria changes in haematological parameters likely to be influenced including endemic diseases that can affect the health of people with various clinical manifestations. Especially in the tropical areas of the world Malaria is a major

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Original Article

Study of Hematological Profile of Adults Presenting with Pancytopenia in a Tertiary Care Hospital of Central India

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Abstract

Background: Pancytopenia is defined as the simultaneous presence of anemia, leukopenia, and thrombocytopenia. The present study was undertaken to explore various causes and clinical manifestations of pancytopenia and to correlate them with severity of pancytopenia in adult patients of Malwa region of central India. **Materials and Methods:** The study was conducted in Department of Pathology of our Institute with the help of clinical departments such as medicine, surgery, oncology, and others. Two hundred and fifty-one admitted adult male and female patients from October 2015 to March 2017 (18 months) presenting with pancytopenia were included in the study. Tests for complete blood count, peripheral smear, reticulocyte count, bleeding time, clotting time, bone marrow aspiration, and trephine biopsy were done. **Results:** Among all the hematological disorders (202/251) causing pancytopenia, the most common was megaloblastic anemia (98/202, 48.51%) followed by dimorphic anemia (36/202, 17.8%) and aplastic anemia (18/202, 8.9%). Least common causes included hemolytic anemia (2/202) and disseminated intravascular coagulation (2/202), that is, 0.99% each. **Conclusion:** Thus, a comprehensive, clinical, and hematological study of patients with pancytopenia will usually help in identifying the underlying cause. The early detection of the underlying conditions would also help to enhance the prognosis of patients with pancytopenia.

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Keywords: *Anemia, Hematological disorders, pancytopenia*

Introduction

Pancytopenia is an important clinicohematological entity encountered in day-to-day practice. The incidence of different disorders causing pancytopenia is variable according to geographical distribution and genetic variation.[1-4] Physical findings and peripheral blood picture provide valuable information in the workup of pancytopenic patients and help in planning investigations on bone marrow samples.[5] Bone marrow evaluation is an invaluable diagnostic procedure which may confirm the cause of suspected cytopenia.[6] If not diagnosed at an early stage, it can be fatal. The underlying pathology determines the management and outcome of the patients. Thus, the present study was carried out to investigate for and to identify the causes of pancytopenia, to find out the frequency of different causes, to determine the incidence of pancytopenia in relation to gender and age, and to compare findings with those of other similar studies from this part of the world.

Materials and Methods

The present study was conducted after approval from the institutional ethics committee. It was an observational study that included 251 adult patients presenting with pancytopenia in institute from October 2015 to March 2017. A thorough clinical history and general examination findings were noted in all cases. Two milliliters of blood sample was collected in K_3 EDTA vials of all the study cases. Complete blood cell

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Original Article

Clinicopathological Diagnosis of Leprosy: Comparative Evaluation of Three Staining Methods for Acid Fast bacilli in Slit Skin Smears and Biopsy Specimens

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Detection of acid fast bacilli (AFB) in slit smears and histopathological specimens is of paramount importance for diagnosis and classification of leprosy. In this study the results of conventional staining technique for AFB has been compared with modified rapid AFB and Fite Faraco stain in slit skin smears and punch biopsy specimens from clinically diagnosed cases of leprosy. Processed skin biopsies and slit skin smears of 42 patients attending outdoor clinic of a tertiary care centre were stained with three stains viz Fite Faraco, modified Rapid AFB and conventional Ziehl Neelsen staining. According to clinical diagnosis the maximum number of patients belonged to Borderline Tuberculoid leprosy which correlated with histopathological diagnosis of skin biopsy. Clinical and histopathological correlation was not observed in 14/42 cases - histopathological categorization of biopsies from these cases revealed Indeterminate leprosy (9 cases), Tuberculoid Leprosy (2 cases), Borderline Lepromatous (1 case), Histoid Leprosy (1 case) and Mid- Borderline Leprosy (1 case). Maximum positivity for AFB was seen with Fite Faraco staining followed by modified Rapid AFB both in the biopsy specimens and slit skin smears. Fite Faraco staining showed highest sensitivity in both paucibacillary and multibacillary cases followed by modified rapid AFB and conventional AFB staining. Though biopsy and slit skin smears have their individual diagnostic advantages and limitations, biopsy deserves to be viewed as gold standard in case of difficulty in arriving at a confirmed diagnosis. Findings of this study need to be validated in a larger number of leprosy cases at community level studies and correlated with classification currently recommended by WHO and NLEP.

Keywords : Granuloma, Slit Skin Smears, Acid Fast Bacilli, Leprosy, Fite Faraco, Modified AFB staining

Introduction

Despite the efforts of the National Leprosy Eradication Programme (NLEP) strategies and plans the fact remains that India continues to account for 60% of new cases reported globally each year and is among the 22 "global priority countries" that contribute 95% of world numbers of leprosy warranting a sustained effort to bring the numbers down (Lockwood 2002, Gurung et al 2019, Srinivas et al 2002). Clinically, leprosy is

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Open Access Review Article

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Role of the Backbenchers of the Renin-Angiotensin System ACE2 and AT2 Receptors in COVID-19: Lessons From SARS

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Abstract

The novel coronaviruses causing severe acute respiratory syndrome (SARS) and coronavirus disease 2019 (COVID-19) have been shown to utilize angiotensin-converting enzyme 2 (ACE2) as the receptor for entry into the host cells. The involvement of the renin-angiotensin system (RAS) in the evolution and pathogenesis of lung diseases has been implicated in recent years. The two enzymes of RAS, angiotensin-converting enzyme (ACE) and ACE2, serve a contrasting function. ACE helps in the formation of angiotensin II (AGII) from angiotensin I (AGI), and ACE2 cleaves AGI and AGII into AG (1-9) and AG (1-7) respectively. The ACE-induced AGII has vasoconstrictor and pro-inflammatory properties via AT1R, whereas ACE2 has been shown to protect against lung injury. The less spoken about AGII receptor, angiotensin receptor type 2 (AT2R), has anti-inflammatory and anti-fibrotic effects in lung tissue and may be of significance in light of the lung pathology presentation in COVID-19. A review of articles searched in PubMed and peer-reviewed journals of importance was done using search terms “ACE2,” “AT2,” “SARS,” and COVID-19.” Lung involvement in both SARS and COVID-19 has been very severe and suggestive of severe inflammatory and immune reactions. Animal studies have shown that ACE2 and AT2 receptors counter the pro-inflammatory and other effects mediated by angiotensin II by their vasodilator, anti-inflammatory, anti-fibrotic, and anti-proliferative effects. They have been shown to protect against and revert acute lung injuries. The instrumental role of recombinant ACE2, AT2 receptor agonists, and AT1 receptor blockers may be helpful in the treatment of COVID-19.

Categories: Miscellaneous, Infectious Disease, Pulmonology
Keywords: ace2, sars, covid-19, ras (renin angiotensin system), coronavirus, angiotensin receptor type 2 (at2r), angiotensin receptor type 1 (at1r), angiotensin ii (agii), ace (angiotensin-converting enzyme)

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Introduction And Background

The coronavirus 2019 (COVID-19) pandemic has affected over 3 million people and caused more than 2 lac deaths in over 200 countries over a duration of four months. There is no medicine or vaccine to date for treatment and symptomatic management is the present treatment plan. Severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) in the past and now COVID-19 are recurring epidemic threats to humans. This review has been undertaken to search probable modes that can be explored to find a drug treatment that can be used to treat COVID-19 or any similar illness involving mainly the respiratory system. The relatively benign coronavirus causing the common cold has taken a novel form, utilizing newer pathways to enter the host and causing severe disease in some cases. The coronaviruses contain a single-stranded 5’ capped positive-stranded ribonucleic acid (RNA) molecule that ranges from 26 to 32 kb. The viral membrane contains the membrane

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(M) glycoprotein, the spike (S) glycoprotein, the envelop (E) glycoprotein, and the nucleocapsid (N) protein. A member of the renin-angiotensin system (RAS), the angiotensin-converting enzyme 2 (ACE2) protein has been reported to be the entry-point receptor for severe acute respiratory syndrome coronavirus (SARS-CoV) and novel SARS-CoV2 causing COVID-19. The viral S protein interacts with the human ACE2, which is present as a transmembrane protein to gain entry into the host [1-3].

The two enzymes of the renin-angiotensin system (RAS), angiotensin-converting enzyme (ACE) and ACE2 serve contrasting functions. ACE converts angiotensin I (AGI) into angiotensin II (AGII) and AGII has vasoconstrictor and pro-inflammatory properties via its type1 receptor (AT1R). ACE inhibitors, e.g. lisinopril, and AT1R inhibitors, commonly known as angiotensin receptor blockers (ARB) like losartan, are very commonly used drugs for cardiovascular diseases like hypertension and cardiac failure. The involvement of RAS in the evolution and pathogenesis of lung diseases has received attention in recent years [4].

The histopathological reports of post-mortem studies of COVID-19 deaths show diffuse alveolar damage, hyaline membrane deposition, fibrin exudates, and consolidation by fibroblastic proliferation with the extracellular matrix and fibrin-forming clusters in air spaces [5-6]. Post-mortem findings of SARS deaths showed features of diffuse alveolar damage (DAD) with pronounced pulmonary edema and hyaline membrane formation. In some areas, there was interstitial thickening, with mild to moderate fibrosis. [7-8] The ACE homolog ACE2, which is the entry receptor for the virus, is expressed abundantly in type II alveolar cells and may have been a base for the rapid expansion of SARS-CoV and a vicious circle of local alveolar wall destruction, resulting in rapidly progressive severe diffuse alveolar damage [9]. Considering the post-mortem findings of the lung, the same may be true in COVID-19. As opposed to ACE, soluble ACE 2 has been shown to protect against lung injury. The less spoken about angiotensin II receptor type 2 (AT2R) has anti-inflammatory and anti-fibrotic effects in lung tissue and may be of significance in light of the lung pathology presentation in COVID-19. It is imperative to assess the role of ACE2 and AT2R as the natural but forgotten protectors of the alveolar environment, along with ARBs, in severe acute lung disease by the novel coronavirus.

Review

Angiotensin-converting enzyme (ACE) and its homolog (ACE2)

Angiotensin-converting enzyme (ACE) is a dipeptidyl carboxypeptidase expressed predominantly in the lung capillary endothelium, and it converts the decapeptide angiotensin I (AGI) to the octapeptide angiotensin II (AGII). AGII has two main receptors, AT1R and AT2R. Angiotensin II via its AT1R receptor causes vasoconstriction along with many other effects on the kidneys and brain to regulate the body fluid volume and blood pressure. It also has pro-inflammatory properties. A study suggested that the activation of the pulmonary RAS influences the pathogenesis of acute lung injury, acute respiratory distress syndrome, and SARS, as they are attenuated by blocking the renin-angiotensin pathway [10]. Thus, the inhibition of the ACE/AGII/ATR1 axis may be beneficial in the setting of its enhanced activity. Both ACE inhibitors and ARBs are used clinically to block the effects of AGII but the ARBs may have a much greater potential to block the renin-angiotensin system than ACE inhibition because an estimated 40% of AG II is formed via non-ACE pathways (e.g. chymase) in humans [11-12].

In the year 2000, a new homolog of ACE was identified separately by two groups and was named ACE2 [13-14]. ACE2 is a zinc metalloprotease with a 40% identity and a 61% similarity to ACE [13]. ACE2 converts AGI to AG (1-9) and AGII to AG (1-7) by removing one amino acid from each. ACE2 is not inhibited by classical ACE inhibitors like captopril, enalapril, and

lisinopril [14]. Both the authors reported that ACE2 is expressed mainly in the heart, kidney, and testis [13-14]. Later, in a study using immunolocalization of the ACE2 protein, it was found that ACE2 is expressed in many tissues, including type I and type II alveolar pneumocytes, nasal, oral and nasopharyngeal mucosa, enterocytes of the small intestine, endothelial cells and smooth muscles of blood vessels of all the tissues studied, and weakly in bronchial epithelial cells [9].

Role of ACE2 in virus entry and lung protection

Numerous animal studies have been conducted to study ACE2 after the discovery that SARS-CoV used it as a portal for entry. These studies may be projected to COVID-19, as its virus also gains entry through the same receptor. The picture in severe cases in both SARS and COVID-19 is that of acute respiratory distress. It was demonstrated in mice that ACE2 was required for the effective replication of SARS-CoV in vivo as ACE2 knockout mice showed a very low quantity of virus when they were infected with SARS-CoV [15]. On one hand, where ACE2 was proved to be the entry receptor for novel CoV, on the other hand, ACE2 knockout mice showed very severe acute lung injury in aspiration, endotoxin, and peritoneal sepsis-induced acute respiratory distress syndrome (ARDS) models. Loss of ACE2 expression in mutant mice resulted in enhanced vascular permeability, increased lung edema, neutrophil accumulation, and worsened lung function [10]. Thus, ACE2 protects murine lungs from ARDS in ACE2 knockout mice. This protective effect of ACE2 is probably by reducing AGII by breaking it to AG (1-7) [10]. Hence, there is a protective effect of ACE2 in acute lung injury with AGII being the probable target. In another study on mice, downregulation of ACE2 expression by SARS-CoV infections and its spike protein was reported. A study also reported that treatment with exogenous recombinant ACE2 protein improved symptoms of acute lung injury in wild type mice as well as ACE2 knockout mice [16]. Thus, ACE2 has a dual role, one, to support virus entry into the host and, two, to protect against severe lung injury.

In relation to ACE2, the findings of studies comparing viruses NL63 and SARS-CoV are intriguing. NL63 is another human coronavirus that uses the same ACE2 for entry as does SARS-CoV but causes mild respiratory disease. Its interaction with ACE2 is weaker than the SARS-CoV S protein interaction with ACE2. NL63-CoV and SARS-CoV have no structural homology in the receptor-binding domain (RBD), yet the two viruses recognize common ACE2 regions [17]. Whether it is lower-affinity interaction with ACE-2 or the difference in ACE-2 signaling following SARS-CoV and NL63 S protein binding or it is the S protein itself that accounts for the differential pathogenicity of human coronavirus NL63 and SARS-CoV remains to be determined [17-18]. Recently, it has been demonstrated that the COVID-19 CoV binds with ACE2 as avidly as SARS-CoV [19]. In a study comparing the two viruses, it was reported that the SARS-CoV binds more efficiently to ACE2 than NL63 and correlates with ACE2 shedding [20]. But working ahead on these findings, with a revised experimental setup, another study found that the ACE2 shedding with NL63 was robust and depended on the fold increase in viral replication and occurred during the early phase of replication [21]. With these observations, it appears that the severity of the disease depends on the efficiency of the binding of the spike protein with ACE2, as both SARS-CoV and CoV2 have greater affinity than NL63. Secondly, the shedding and downregulation of ACE2 expression does not seem to determine the potential severity of lung disease but is associated with higher viral replication. A study in 2004 found that the soluble ACE2 ectodomain specifically blocked infection by SARS-CoV S-bearing pseudotypes, and this finding may be a breakthrough for COVID-19 also [22]. Thus, recombinant ACE2 may be beneficial in blocking virus entry and in reducing lung injury in COVID-19 via some missing link. Thus the double-edged sword ACE2 must be explored to be used appropriately for conquering COVID-19 by strategic administration through its considered inclusion in the predefined and agreed-on treatment protocol of COVID-19.

Angiotensin II receptor subtype (AT2R)

AGII, the major effector peptide of the RAS, promotes vasoconstriction, proliferation, inflammation, and fibrosis within the pulmonary vasculature and lung parenchyma via stimulation of AT1 receptors. The use of losartan to block the AT1 receptor improved lung injury in this mouse model. The aforementioned detrimental effects of AGII may be counterbalanced by the activation of AT2 receptors [23].

AT2R has not been given much attention after its discovery, as most of the functions of AGII were operated via AT1R and the significance of AT1R in cardiovascular and renal pathologies became well-known. The expression of AT2R in healthy adults is often low and is mainly identified in the renal, cardiovascular, adrenal medulla, brain tissues, myometrium, and ovaries [24-25] In contrast, in fetal tissues, AT2R is the dominant receptor subtype [26]. In the adult, AT2Rs are re-expressed or upregulated under certain pathophysiological conditions, such as mechanical injury or ischemia like myocardial infarction, vascular injury, brain ischemia, and renal failure [24,27]. AT2R exerts vasodilator, anti-fibrotic, and anti-inflammatory effects in a variety of disease models, as well as natriuretic and antihypertensive effects in renal disease [28]. The AT2R-specific agonist may effectively dampen the pro-inflammatory and aggressive behavior in rheumatoid synovitis [29]. Thus, AT2R has proven anti-inflammatory effects in a variety of tissues. The activation of AT2R is thought to counter-regulate the pathophysiological effects of angiotensin II (AGII) induced by AT1R [24].

AT2R research gained momentum when the first nonpeptide AT2R agonist, compound 21 (C-21) was introduced in 2004 [24]. A study investigated the effect of the AT2R agonist C-21 in the bleomycin model of pulmonary fibrosis and reported the beneficial effects of C-21, which were associated with decreased infiltration of macrophages in the lungs, reduced lung inflammation, and diminished pulmonary collagen accumulation [30]. In the monocrotaline (MCT)-induced pulmonary hypertension rat model, C-21 treatment reversed both interstitial and perivascular fibrosis. Furthermore, a decrease in ACE2 messenger RNA (mRNA) levels was observed in MCT-induced pulmonary hypertension animals, which was reversed by C-21 therapy with a two-fold increase in ACE2 levels and a concomitant decrease in ACE expression [23]. The protective effect of ACE2 has already been emphasized. Furthermore, C-21 treatment normalized the AT1/AT2 receptor ratio to restore the lung RAS balance. C-21 treatment prevented, as well as attenuated, the progression of lung fibrosis and the accompanying pulmonary hypertension. Thus, in view of the above inferences, the acute inflammatory lung pathology being observed in COVID-19 may be ameliorated by the AT2R agonist.

Conclusions

Three classes of drugs affecting RAS may be candidates to ameliorate the acute lung pathology in COVID-19. With the possibility of AGII overexpression as the trigger in COVID-19 lung pathology, its inhibition may be of benefit. Here, ARBs like losartan may be more beneficial than ACE inhibitors for the fact that sufficient AGII is formed by ACE non-dependent mechanisms. CoV2 uses ACE2 to gain entry into the host, and after gaining entry, it causes the shedding and downregulation of ACE2 to sabotage its anti-inflammatory and protective effect. This, along with the observation that the soluble ACE2 ectodomain specifically blocked infection by SARS-CoV S-bearing pseudotypes, shows that the use of soluble recombinant ACE2 may be helpful in reducing lung injury in COVID-19. Our speculation is that AT2R may be re-expressed in COVID-related lung injury and hence the use of AT2R agonists may also be explored to elicit their anti-inflammatory and anti-fibrotic effects. Bacterial communicable diseases were a menace and created havoc prior to the antibiotic era. However, during the last few decades, public health focus has largely been on non-communicable diseases and related lifestyle interventions. The sudden spurt of COVID-19 and its global spread demands innovative approaches using forgotten enzymatic pathways, as suggested in this study, for magnanimous improvements in the clinical outcome among the COVID-19 cases. The herein suggested interventions with ACE2 and AT2 receptors agonist, along with ARBs, may be

beneficial in improving the clinical outcome of COVID-19.

Additional Information

Disclosures

Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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Public Health Threat Assessment of Vehicular Load Index-Induced Urban Air Pollution Indices Near Traffic Intersections In Central India

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Abstract

Objectives: To assess traffic vehicular load, levels of various air pollutants, their correlation at selected traffic intersections of Bhopal city and to suggest suitable public health measures.

Methods: A transverse study was conducted by convenience sampling with equated distribution among vehicular load-based large (Group1:G1: 10 TI), medium (Group2:G2: 5 TI), and small (Group3:G3: 5 TI) traffic-intersections (TI) through a systematic stratified random selection of study sites to assess traffic vehicle load index (VLI).

Results: VLI,G1 (cumulative mean: 16.31; day-time (DT): 19.03, DT range 11.68-51.49; night-time (NT): 13.59, NT range 11.7-18.0), VLI,G2 (cumulative mean: 0.965; DT:0.971, DT range 08.56-11.67; NT: 0.960, NT range 07.54-11.39), and VLI,G3 (cumulative mean: 06.17; DT:06.08, DT range 04.12-06.86; NT: 06.27, NT range 03.74-07.53). There is a significant intergroup difference of the mean (G1 vs G2: p=0.03); (G1 vs G3: p=0.002); (G2 vs G3: p=0.003). The range of VLI is found to be wide within G1 (DT; 11.68-51.49; NT 11.7-18.00) as compared to narrow range in G2 (DT; 8.56-11.67; NT7.54-11.39) and G3 (DT; 4.12-6.86; NT 3.74-7.53).

Conclusion: High air pollution noted at TIs and associated exposure to unprotected commuters pose public-health risks. It has long-term health consequences requiring focused multidisciplinary preventive interventions.

Categories: Preventive Medicine, Public Health, Other
Keywords: air pollution, assessment, particulate matter, public health, traffic

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Introduction

Air pollution has been posing long-term, medium-term, and short-term challenges since long to the public health authorities thereby adversely affecting air quality indices [1]. PM10 standards for the ecologically sensitive area as per National Ambient Air Quality Standards is 60 µg/m^3 for an annual time-weighted mean of a minimum 104 measurements in a year at a particular site taken twice a week, 24 hourly at uniform intervals. PM10 standards for 24 hours time-weighted mean is 100 µg/m^3. There is a felt need to revise PM10 standards to ensure avoidance of air pollution through preventive interventions at multiple levels with a special focus on maternal and child health [2].

Many studies have conducted air pollution indices in an indoor environment, but the studies related to the outdoor environment are much needed [3-8]. In 2015, Government of India, together with IIT Kanpur launched the National Air Quality Index. In 2019, India launched "The National Clean Air Programme" with a tentative national target of 20-30% reduction in PM2.5 and PM10 concentrations by 2024, considering 2017 as the base year for comparison. It will be rolled out in 102 cities that are considered to have air quality worse than the National Ambient Air Quality Standards. The present study will identify the traffic load wise pollution indices and their interrelationship. Hence, this study shall enrich the understanding of the vehicular pollution load in Central India as per the important pollution indicators.

We hypothesize that the analysis of various pollutants viz. (PM 2.5, TVOC, CO_2, HCHO, PM1.0, PM 5.0, PM 10, and submicron particles) influence outdoor air quality. These pollutants are a threat for enhanced health risks especially for the vulnerable population including the elderly, children, and pregnant women. Therefore, the purpose of this study is to assess traffic vehicular load, levels of various air pollutants, and their correlation at selected TIs of Bhopal city and to suggest suitable public health measures. This study will also provide recent most air pollution scenario in moderately populated Bhopal city for possible timely preventive interventions.

Materials And Methods

A transversal study was conducted at a Tertiary Health Care Centre, Bhopal, India from June to September, 2019 to assess traffic vehicular load, levels of various air pollutants and their correlation at selected TIs of Bhopal city, and to suggest suitable public health measures.

Sample collection

The systematic stratified random selection of the TIs was conducted by convenience sampling with equated distribution among load-based large (Group1-G1), medium (Group2-G2), and small (Group3-G3) TIs (total 20). Vehicle load index (VLI) was assessed using "vehicular indices calculation matrix datasets." The vehicle load was determined herein by the exclusive VLI developed in this study assuming the load of each two-wheeler, three-wheeler, cars, medium vehicle, and the heavy vehicle being 1.0, 1.5, 2.0, 2.0, and 6.0, respectively. The number of vehicles passing through each studied TIs was assessed lane wise as a mean of three readings at day-time (DT) and night-time (NT) for three days inclusive of last day being the reading date for all the air pollutants using calibrated Ambee Air Quality Monitor TM. The total number of TIs (n=20) included in the study were distributed in high traffic areas (G1: n=10), medium traffic area (G2: n=05), and low traffic area (G3: n=05). These included signaled (S) TIs (G1:07; G2:05; G3:01), semi-signaled (SS) TIs (G1:01; G3:01), non-signaled (NS) TIs (G1:02; G3:03S) (Tables *1* and 2).

S.No.	TI sites (S/SS/NS) #	VLI		TI site mean	TI group mean		
		DT	NT		DT	NT	Overall mean
1	Arera colony (S)	51.49	18.00	34.74	19.03	13.59	16.31
2	Chetak bridge (S)	19.76	17.23	18.49			
3	New market (S)	18.83	13.97	16.4			
4	Shivaji nagar (S)	16.94	13.34	15.14			
5	Anand nagar (NS)	15.53	12.67	14.1			
6	Old city (NS)	15.01	12.48	13.7			
7	Ambedkar Square (S)	14.52	12.3	13.4			
8	Lalghati (S)	14.24	12.28	13.26			
9	Piplani (SS)	12.34	11.94	12.14			
10	Polytech (S)	11.68	11.7	11.69			
G2: Total 05 TIs: Medium traffic areas							
11	Bus st. (S)	11.67	11.39	11.53	09.71	09.60	09.65
12	Karond (S)	09.98	10.1	10.04			
13	Chowk (S)	09.29	09.69	09.54			
14	Habibganj (S)	09.07	09.31	08.155			
15	Railway station (S)	08.56	07.54	08.05			
G3: Total 05 TIs: low traffic areas							
16	Gandhi nagar (S)	06.86	07.53	07.19	06.08	06.27	06.17
17	Mandi (SS)	06.83	06.88	06.85			
18	Narela (NS)	06.55	06.83	06.69			
19	Ayodhya (NS)	06.07	06.38	06.22			
20	Kasturba (NS)	04.12	03.74	03.93			

TABLE 1: VLI in the DT and NT at TIs of groups G1, G2, G3 in Bhopal city during June-September, 2019 (n=20)

#(S)=signaled TI: (NS)=non-signaled TI: (SS)= semi-signaled TI.

TI: traffic intersection, VLI: vehicle load index, DT: daytime, NT: nighttime.

Groups	VLI		TVOC		CO_2		HCHO		PM10		Submicron particles (>0.25, >0.3, >0.5, >10) present at (no. of TI/total TI)
	DT:NT ratio	Group mean	DT:NT ratio	Group mean	DT:NT ratio	Group mean	DT:NT ratio	Group mean	DT:NT ratio	Group mean	
G1	1.40	16.31	2.45	0.73	1.30	1170.75	2.69	1.91	2.50	320.03	47/50
G2	1.01	9.65	0.52	1.40	1.19	921.90	0.75	0.14	0.09	43.44	24/25
G3	0.96	6.17	3.66	0.16	1.18	830.30	3.00	0.02	0.53	145.97	23/25

TABLE 2: Group-wise comparison of VLI with gaseous particles and particulate matter (n=20)

TI: traffic intersections, VLI: vehicle load index, TVOC: vehicle load index, DT: daytime, NT: nighttime.

Measurement of pollution parameters

Various pollutants PM2.5, TVOC, CO_2, HCHO, PM1.0, PM5.0, PM10, particle size >0.3 µm, >1.0 µm, >2.5 µm, >5.0 µm, >10 µm, temperature, dew point, humidity, ultraviolet, and visibility were recorded in DT and NT at all selected TIs using calibrated Ambee Air Quality Monitor™ (Ambee Pvt., Ltd., Mumbai, IND). Hence, the study variables included particulate matter: PM (PM 2.5, PM10, PM1, PM5); gaseous pollutants (CO_2, HCHO, TVOC); submicron particles (>0.25 µm, >0.3 µm, >0.5 µm, >10 µm). In addition to the above, the meteorological conditions prevailing on the days of data collection included temperature, humidity, dew, wind speed, air pressure, ultraviolet radiation, and visibility. The inclusion criteria for the study were randomly selected ten large, five medium, and five small traffic transactions. The convenient sampling was done twice a day for three consecutive days at each of the randomly selected sites. The list of these categories was prepared based on the pilot study undertaken for the overall load of traffic transactions at ten intersections of Bhopal distributed in all of its geographical zones. Those not listed in the randomly selected list of traffic transactions were excluded from the study. The portable digital device used for assessing various pollution parameters in the study was Ambee Air Quality Monitor™.

Study sites

Twenty TIs were chosen in Bhopal city as per inclusion and exclusion criteria. Based on the traffic load, these TIs were divided into large, medium, and small. Various atmospheric pollutants studied included PM 2.5, PM 10, HCHO, CO_2, etc., were monitored using Ambee Air Quality Monitor™. Vehicle at the intersections was further divided into two-wheeler, three-wheeler, cars, heavy, and medium vehicles. Traffic load at each lane of each of the TIs was calculated for three consecutive days with the last day being data collection day for meteorological and air pollution parameters as well. The VLI was assessed as described above. The study was carried out at DT from peak traffic hours of 10 AM to 12 PM and at night from 6 PM to 8 PM. Random quick interviews were also conducted among pollution under control (PUC) check service provider, car service agencies, roadside tyre puncture repair shops, and traffic constables in each of these categories (G1, G2, G3).

Statistical analysis

All data entered were analyzed using SPSS (Statistical Package for Social Science) Version 20 (IBM Corp, Armonk, USA).

Results

Cumulative values of VLI

VLI,G1 (cumulative mean: 16.31; DT: 19.03, DT range 11.68-51.49; NT: 13.59, NT range 11.7-18.0), VLI,G2 (cumulative mean: 0.965; DT: 0.971, DT range 08.56-11.67; NT: 0.960, NT range 07.54-11.39), and VLI,G3 (cumulative mean: 06.17; DT: 06.08, DT range 04.12-06.86; NT: 06.27, NT range 03.74-07.53). The TI site mean for G1 ranged 11.69-34.74, whereas the same for G2 and G3 were 8.05-11.53 and 03.93-07.19, respectively (Table *1*). There is significant intergroup difference of the mean (G1 vs G2: p=0.03); (G1 vs G3: p=0.002); (G2 vs G3: p=0.003) (Table *3*) The range of VLI is found to be wide within G1 (DT: 11.68-51.49; NT: 11.7-18.00) as compared to narrow range in G2 (DT: 8.56-11.67; NT: 7.54-11.39) and G3 (DT: 4.12-6.86; NT: 3.74-7.53).

S. No.	Description	Group	Spearman correlation (rs)	p-value (two-tailed)	Significance
1	VLI vs PM2.5	G1	-0.46	0.17	-
		G2	-0.6	0.28	-
		G3	0.21	0.73	-
		G1+G2+G3	-0.43	0.05	+
2	VLI vs PM10	G1	0.12	0.72	-
		G2	-0.8	0.1	-
		G3	0.79	0.11	-
		G1+G2+G3	-0.14	0.55	-
3	PM2.5 vs PM10	G1	0.28	0.42	-
		G2	0.9	0.03	+
		G3	0.4	0.5	-
		G1+G2+G3	0.49	0.02	+
4	PM1.0 vs PM10	G1	0.87	0.00	+
		G2	0.8	0.1	-
		G3	0.5	0.39	-
		G1+G2+G3	0.71	0.00	+
5	PM1.0 vs PM2.5	G1	0.53	0.1	-
		G2	0.9	0.03	+
		G3	0.6	0.28	-
		G1+G2+G3	0.59	0.01	+
6	PM10 vs TVOC	G1	-0.04	0.9	-
		G2	0.1	0.87	-
		G3	-0.35	0.55	-
		G1+G2+G3	-0.25	0.27	-
7	PM10 vs CO_2	G1	0.21	0.55	-
		G2	0.1	0.87	-
		G3	0.1	0.87	-
		G1+G2+G3	0.33	0.15	-
8	PM10 vs HCHO	G1	0.5	0.13	-
		G2	-0.66	0.21	-
		G3	-0.22	0.71	-
		G1+G2+G3	0.05	0.82	-

TABLE 3: Intragroup and intergroup Spearman's correlation coefficient for the observed noticeable values of importance (PM10, PM2.5, PM1, TVOC, CO2, HCHO, and VLI)

(-) = not significant; (+) = significant.

TVOC: total volatile organic compounds, PM: particulate matter, VLI: vehicle load index.

Measuring air pollution indices

The air pollution indices measured at DT and NT included gaseous particles (TVOC, CO_2, HCHO) particulate matter (PM1.0, PM2.5, PM5, PM10) and submicron particles (>0.3 µm, >1.0 µm, >2.5 µm, >5.0 µm, >10 µm) at all 10, 05, and 05 TIs of G1, G2, and G3, respectively. The group-wise range of values for air pollution indices are indicated for DT and NT pollution level (Tables *4* and 5).

S No.	TIs	VLI	PM 2.5	TVOC	CO_2	HCHO	PM 1.0	PM 5.0	PM 10	>0.3 µm	>1.0 µm	>2.5 µm	>5.0 µm	>10 µm
1	Arera colony	51.49	26.1	1.02	1121	16.7	31	38.2	2720	320	112	30	10	3
2	Chetak bridge	19.76	7.1	1.06	4043	0.15	3.77	8.15	8.3	710	198	19	6	2
3	New market	18.83	1.35	0.17	1182	9.81	17.4	18.2	1617	189	56	18	7	5
4	Shivaji nagar	16.94	21.12	1.61	627	0.23	0.97	1.25	2.09	24	9	0	0	0
5	Anand nagar	15.53	41.6	0.09	996	0.01	18.8	45.1	45	2720	540	175	56	28
6	Old city	15.01	0.48	0.09	737	0.01	0.32	0.41	0.42	115	1	0	0	0
7	Ambedkar Square	14.52	43	1.61	1346	0.23	37.5	80.3	87.9	6568	854	244	72	36
8	Lalghati	14.24	63.3	0.16	568	0.02	12.5	37	31.7	4170	262	59	16	4
9	Piplani	12.34	36.2	0.5	2047	0.07	16	36	35.3	2765	5512	144	51	19
10	Polytech	11.68	30.8	4.08	578	0.58	10.8	25.6	28.9	2081	322	109	28	8
11	Bus stand	11.67	40.8	0.42	767	0.06	37.4	53.6	54.3	6023	505	59	15	0
12	Karond	9.98	26.1	0.42	2555	0.06	12.5	37.6	44.5	2207	497	209	61	38
13	Chowk	9.29	21.3	1.34	714	0.1	0.09	1.33	0.66	54.2	15	10	0	1
14	Habibganj	9.07	0.11	2.53	946	0.36	0.14	0.38	0.4	38	4	2	0	0
15	Railway station	8.56	27.4	0.22	648	0.03	16	21.9	21.5	2698	151	10	0	0
16	Gandhi nagar	6.86	37.4	0.09	578	0.01	20.3	44.4	46.1	2970	599	111	17	1
17	Mandi	6.83	22.5	0.3	1225	0.04	14.6	46.4	50.3	3026	727	337	126	54
18	Narela	6.55	32.3	0.09	727	0.01	154	35.9	37.7	2584	440	94	9	6
19	Ayodhya	6.07	5.51	0.56	797	0.08	4.45	11	11.8	758	112	43	3	0
20	Kasturba	4.12	30.2	0.1	878	0.01	0.06	17.8	32.3	140	327	109	16	0

TABLE 4: Air pollution indices at DT (10 AM to 12 PM) during June-September, 2019 in Bhopal city (n=20)

TI: traffic intersection, TVOC: total volatile organic compounds, VLI: vehicle load index.

S. No.	TIs	VLI	PM 2.5	TVOC	CO_2	HCHO	PM 1.0	PM 5.0	PM 10	>0.3 µm	>1.0 µm	>2.5 µm	>5.0 µm	>10 µm
1	Chetak bridge	18	8.75	0.86	1651	0.12	6.57	10.5	10.7	1350	120	26	4	0
2	Anand nagar	17.23	32.4	0.35	977	0.05	17	40.4	42.9	2790	520	133	39	15
3	Shivaji nagar	13.97	12.3	1.35	1176	0.19	9.81	17.4	18.2	1617	189	56	10	2
4	New market	13.34	1.35	o.19	1176	9.81	17.4	18.2	1617	189	56	18	7	0
5	Piplani	12.67	72.9	0.79	1186	0.11	33.3	74.2	87.9	6637	940	374	141	53
6	Lalghati	12.48	10.4	0.09	648	0.01	6.15	12.7	13.3	1080	165	37	11	9
7	Ambedkar square	12.3	4.5	0.07	578	0.01	9.11	13.87	14.02	187	32	1	0	0
8	Old city	12.28	37.2	0.04	865	0.02	3.71	5.1	0.33	108	1	0	0	0
9	Polytech	11.94	2.96	0.09	568	0.01	0.74	1.24	1.25	200	12	0	0	0
10	Arera colony	11.7	23.1	0.43	1345	0.06	10.5	17.9	18.5	1712	181	24	3	2
11	Karond	11.39	39	0.07	638	0.01	8.84	20.2	43.7	1385	294	78	21	54
12	Bus stand	10.1	27.8	6.11	875	0.54	22.5	28.9	43	1345	321	35	8	4
13	Habibganj	9.69	14.3	0.42	1440	0.03	10.6	19.2	20.3	1990	212	58	10	2
14	Chowk	9.31	19.38	2.41	738	0.21	0.11	1.03	1.01	57	11	14	10	0
15	Mandi	7.54	28.9	0.27	1022	0.02	11.4	50.2	1151	642	449	126.4	42	12
16	Gandhi nagar	7.53	28.3	0.05	607	0.01	30.2	247	58.7	1990	628	79	11	0
17	Railway station	6.88	22.3	0.1	392	0.01	17	20	205	2440	134	11	1	0
18	Ayodhya	6.83	12.1	0.1	732	0.01	7.9	8.9	7.2	1101	38	0	0	0
19	Narela	6.38	29.9	0.01	805	0.01	11.4	28.9	40.4	977	374	71	11	2
20	Kasturba	3.74	27.8	0.08	932	0.01	0.03	0.16	24.2	116	242	98	10	0

TABLE 5: Air pollution indices at NT (6 PM to 8 PM) during June-September, 2019 in Bhopal city (n=20)

TI: traffic intersection, TVOC: total volatile organic compounds, VLI: vehicle load index.

Cumulative range values of gaseous particles, particulate matter, and submicron particles

The cumulative range values of DT and NT were also assessed (Table 5). The noticeably high-value levels among groups (G1, G2, G3) were found for the following parameters (as per observed TI/total TI: G1: TVOC - 7/10; CO_2 - 8/10; HCHO - 6/10; PM10 - 2/10, and submicron particles at all sites for all sizes ranging >0.3 µm to >10 µm except at 3/50 readings; G2: TVOC - 3/5; CO_2 - 4/5; HCHO - 3/5; PM10- 1/5, and submicron particles at all sites for all sizes ranging >0.3 µm to >10 µm except at 1/25 readings; G3: CO_2 - 4/5; PM10 - 1/5 and submicron particles at all sites for all sizes ranging >0.3 µm to >10 µm except at 2/25 readings). It is further observed from group-wise data that CO_2 level is found increased in all the groups almost at all sites except 4/20, whereas TVOC and HCHO are not found increased in G3. TVOC and HCHO are found generally raised in G1 and G2 with cumulative non-observance in 5/15 sites and 6/15 sites, respectively. The TIs with the market area have essentially shown PM10 to be high irrespective of group categorization of the concerned site (Table 6).

TIs	VLI	PM 2.5	TVOC	CO_2	HCHO	PM 1.0	PM 5.0	PM 10	PM2.5/PM10	>0.3 µm	>1.0 µm	>2.5 µm	>5.0 µm	>10 µm
Arera colony	31.5	24.6	0.72	1233	8.38	24.2	28.05	1369.25	0.87	6	146.5	27	6.5	2.5
Chetak bridge	18.85	7.9	0.96	2847	0.13	5.17	9.325	9.5	0.84	1030	159	22.5	5	1
New market	16.08	1.35	0.17	1179	9.81	17.4	18.2	1617	0.07	189	56	18	7	2.5
Shivaji nagar	15.4	16.7	1.48	901.5	0.21	5.39	9.325	10.14	1.79	820.5	99	28	5	1
Anand nagar	14.1	37	0.22	986.5	0.03	17.9	42.75	43.95	0.86	2755	530	154	47.5	21.5
Old city	13.6	18.6	0.06	801	0.01	2.015	2.755	0.37	6.75	111.5	1	0	0	0
Ambedkar Square	13.4	23.7	0.84	962	0.12	23.305	47.085	50.96	0.5	3377.5	443	122.5	36	18
Lalghati	13.3	36.8	0.12	608	0.01	9.325	24.85	22.50	1.48	2625	213.5	48	13.5	6.5
Piplani	12.5	54.5	0.64	1616.5	0.09	24.65	55.1	61.60	0.98	4701	3226	259	96	36
Polytech	11.8	21.3	2.08	573	0.295	5.77	13.42	15.07	1.58	1140.5	167	54.5	14	4
Group mean: G1: [(TVOC:0.73); (CO_2: 1170.75); (HCHO:1.91); (PM10:320.03)]														
Bus stand	10.8	34.3	3.26	821	0.30	29.95	41.25	48.65	0.83	3684	413	47	11.5	2
Karond	10.6	32.5	0.24	1596.5	0.03	10.67	28.9	44.10	1.12	1796	395.5	143.5	41	46
Chowk	18.3	20.3	1.87	726	0.15	0.1	1.18	0.83	17.2	55.6	13	12	5	0.5
Habibganj	18.32	7.2	1.47	946	0.19	5.37	9.79	10.35	0.73	1014	108	30	5	1
Railway street	7.7	24.8	0.16	520	0.02	16.5	20.95	113.25	1.18	2569	142.5	10.5	0.5	0
Group mean: G2: [(TVOC: 1.40); (CO_2: 921.90); (HCHO: 0.14); (PM10:43.44)]														
Gandhi nagar	7.1	32.8	0.07	592.5	0.01	25.25	145.7	52.40	0.22	2480	613.5	95	14	0.5
Mandi	7.1	25.7	0.28	1123.5	0.03	13	48.3	600.65	0.53	1834	588	231.7	84	33
Narela	6.4	31.1	0.05	766	0.01	82.7	32.4	39.05	1.04	1780.5	407	82.5	10	4
Ayodhya	6.4	8.8	0.33	764.5	0.04	6.175	9.95	9.50	1.13	929.5	75	21.5	1.5	0
Kasturba	3.9	29	0.09	905	0.01	0.045	8.98	28.25	3.22	128	284.5	103.5	13	0
Group mean: G3: [(TVOC: 0.16); (CO_2: 830.30); (HCHO: 0.02); (PM10:145.97*)]														
Cumulative mean of all TIs: (G1+G2+G3): [(TVOC:0.75); (CO_2: 1023.42); (HCHO: 0.99); (PM10: 207.37)]														

TABLE 6: Cumulative values of DT and NT gaseous particles, particulate matter, and submicron particles against observed vehicular load as assessed by VLI in Bhopal city during June-September, 2019 (n=20)

TI: traffic intersection, TVOC: total volatile organic compounds, VLI: vehicle load index.

TVOC (cumulative 0.75; range 0.05-3.26; G1: 0.73; G2: 1.40; G3: 0.16); CO_2 (cumulative 1023.42; range 520-2847; G1: 1170.75; G2: 921.90; G3: 830.30); HCHO (cumulative 0.99; range 0.01-9.81; G1: 1.91; G2: 0.14; G3: 0.02); PM10 (cumulative 207.37; range 0.37-1617; G1: 320.03; G2: 43.44; G3: 145.97; Table *6*). However, PM2.5 was acceptable in most groups. Particle size >0.3 µm, >1.0 µm, >2.5 µm, >5.0 µm, and >10 µm were

high. The 95% confidence interval of individual sample mean G1 (12.1016-20.5184), G2 (7.8580-11.442), G3 (4.5519-7.7880) is also depictive of calculated significant p-value (0.001365). The 95% confidence interval assuming equal variance for G1 (13.17-19.44), G2 (4.20-15.09), G3 (0.72-11.61) has F-statistics of value 9.9695 (Table 7). The significance of inter group difference of the mean is observed as: (G1 vs G2: p=0.03); (G1 vs G3: p=0.002); (G2 vs G3: p=0.003) (Table 3).

TI groups	Sample size (n=20)	Mean + SD	SE	95% CI of the individual sample mean	95% CI assuming equal variance	F-statistics	p-value
G1	10	16.31 + 5.88	02.11	12.10 - 20.51	13.17 - 19.44	9.9695	0.001365
G2	05	09.65 + 1.44	00.64	07.85 - 11.44	4.20 - 15.09		
G3	05	06.17 + 1.30	00.58	04.55 - 7.78	0.72 - 11.61		

TABLE 7: ANOVA of VLI for comparison within and among the groups (n=20)

ANOVA: analysis of variance, CI: confidence interval, VLI: vehicle load index, SE: standard error.

Intragroup correlation

The intragroup Spearman's rank correlation coefficient is found to be significant in some groups (PM2.5 vs PM10: (G2: 0.03)]; PM1.0 vs PM2.5: (G2: 0.03)]. The intergroup Spearman's rank correlation coefficient was found significant for cumulated group TIs among some sites [VLI vs PM2.5 (rs= -0.43; p=0.05); PM2.5 vs PM10 (rs= 0.49; p=0.02); PM1.0 vs PM10 (rs=0.71; p=0.00); PM1.0 vs PM2.5 (rs=0.59; p=0.01). The intergroup Spearman's rank correlation coefficient for cumulated group TIs among remaining sites was found non-significant for groups namely VLI vs PM10, PM10 vs TVOC, PM10 vs CO_2 and PM10 vs HCHO (Table 3).

Discussion

Our study observed range of site mean for G3 (3.93-7.19), G2 (8.05-11.53), G1 (11.69-34.74) are consecutively ascending in nature similar to the observed DT (G3-6.08, G2-9.71, G1-19.03), night time (G3-6.27, G2 -9.60, G1-13.59), and overall group mean (G3-6.17, G2-9.65, G1-16.31) (Table 1). Similarly, a Madurai-based study 17 [9] has specifically underlined the importance of PM10 for vehicle-related pollution. The present study has also found highly increased PM10 in some of the TIs of G1, G2, and G3 groups [(G1/1: PM10, 1369.25); (G2/5: PM10, 113.25), and (G3/2: PM10, 600.65); Table 6]. While this study assessed VLI also as an indicator of traffic intersection-wise pollution (Tables 1, 2, and 7). Similarly, another study [10] conducted during February-March 2012 at Bhopal assessed PM10 and PM2.5 beyond permissible limits but SO_2 and NO_2 were within prescribed limits (AQI 105.54, February; AQI 105.89, March). However, the VLI was not assessed therein. The present study has been conducted at different locations of the city. However, the seasonal variation, large scales gatherings, and the annual increase in vehicular pollution due to the use of old vehicles may be limitations of the study.

The range of mean difference for PM10 was 7.2-12.7 $\mu g/m^3$, whereas the mean difference for PM2.5 was 7.9 $\mu g/m^3$ in a study conducted by the Department of Air Quality at The Netherlands [11] indicating 1.3 times higher concentration than the background levels. This study observed group mean for PM10 in descending order among G1, G2, G3 (PM10: (G1: 319.87; G2: 138.00; G3: 51.37) and DT:NT for PM10 for the same order is noted as 2.50, 0.09, and 0.53 (Table 2). It hence shows that, despite the rainy season during the present study, the indicators of air pollution in the city of Bhopal are high as evidenced by high TVOC, CO_2, HCHO, and PM10 levels of cumulative datasets (Table 6), DT (Table 4), and NT (Table 5) for gaseous particles, particulate matter, and submicron particles assessed during June-September, 2019.

PM10 concentration was found to be raised due to local vehicular traffic in Finland in a study conducted by the Finnish Meteorological Institute [12], whereas PM2.5 concentration was assessed to be high at highways-based transport regions. The present study similarly infers that the increasing traffic load across traffic intersections in the city with expanding developmental initiatives under Capital Development Projects and other private developers shall pose further threats of higher air pollution levels in the years to come with seasonal variations challenging the public health scenario.

Seasonal trends of summer, autumn, winter were assessed in the Republic of Korea [13] by Korea Railroad Research Institute and found significantly high values of pollution indices viz. PM10 (42.5-108.4 $\mu g/m^3$),

PM2.5 (61.1-64.0 µg/m^3), PM2.5/PM10(0.60), PM1.0(50.9- 52.2 µg/m^3), PM1/PM2.5 (0.79-0.85), and CO_2 (686.9-701.5 ppm). These findings are in resonance with the findings of the present study. A study in Seoul Metropolitan Subway Stations at Han Yang University, Seoul [14] similarly found PM10 and PM2.5 to be higher than permissible levels of 150 µg/m^3 and 35 µg/m^3, respectively, which were significantly higher than those at ground level ($p<0.05$). These Korean studies highlight the need to generate evidence of air pollution by on-road vehicles and related adverse consequences on human health across the spectrum of demographic profiles. The University of Porto, Portugal [9] collected data of DT and NT for PM10, PM2.5, and PM1.0 and found these to be on a higher level with PM10 (DT mean 125+73 µg/m^3; NT mean 110+71 µg/m^3), PM2.5 (DT mean 115+68 µg/m^3; NT mean 108+70 µg/m^3), and PM1.0 (DT mean 114+68 µg/m^3; NT mean 107+69 µg/m^3). Seasonal trends of summer, autumn, winter were assessed in the Republic of Korea [7] by Korea Railroad Research Institute and found significantly high values of pollution indices, viz., PM10 (42.5-108.4 µg/m^3), PM2.5 (61.1-64.0 µg/m^3), PM2.5/PM10 (0.60), PM1.0 (50.9- 52.2 µg/m^3), PM1/PM2.5 (0.79-0.85), and CO_2 (686.9-701.5 ppm). These findings are in resonance with the findings of the present study. These Korean studies highlight the need to generate evidence of air pollution by on-road vehicles and related adverse consequences on human health across the spectrum of demographic profiles. The University of Porto, Portugal [15] collected data of DT and NT for PM10, PM2.5, and PM1.0 and found these to be on a higher level with PM10 (DT mean 125+73 µg/m^3; NT mean 110+71 µg/m^3), PM2.5 (DT mean 115+68 µg/m^3; NT mean 108+70 µg/m^3), and PM1.0 (DT mean 114+68 µg/m^3; NT mean 107+69 µg/m^3).

A study conducted at Central Road Research Institute, New Delhi [16] identified PM, VOC, and gas chemicals to be hazardous air pollutants in indoor and outdoor sources. As per sampling done by Grimm Dust Monitor and VOC Monitor at sampling time between 9:30 am and 5:00 pm while noting even the corridor air pollutants level to be alarming in office buildings (PM10: 83.4+44.7 µg/m^3, PM2.5 65.0+37.3 µg/m^3, PM1.0 57.8+29.9 µg/m^3) and VOCs (64.4+21.6 ppm). PM10 concentrations at schools located in city center, residential, and rural area with three classrooms in each (total nine measurement sites) observed PM10 during occupancy to be as (a) school 1: [site 1: (PM10 81.0+11.7 µg/m^3); site 2: PM10 104+63.5 µg/m^3); site 3: PM10 70.1+25.2 µg/m^3)]; (b) school 2: [site 1: (PM10 97.0+15.9 µg/m^3); site 2: PM10 362+83.7 µg/m^3); site 3: PM10 177.1+75.0 µg/m^3)]; (c) school 3: [site 1: (PM10 68.0+19.6 µg/m^3); site 2: PM10 73.7+13.8 µg/m^3); site 3: PM10 62.2+8.93 µg/m^3)] were observed in a study [17]. A study conducted at Indian Institute of Technology, Kharagpur in association with West Bengal Pollution Control Board [18] assessed PM10 and PM2.5 at three sites in Kolkata and observed similar findings [PM2.5 site 1: (96.31-355.19 µg/m^3); site 2: (116.29-363.63 µg/m^3); site 3: (99.14-263.0 µg/m^3) PM10 site 1: (140.5-471.7 µg/m^3); site 2: (216.21-637.7 µg/m^3); site 3: (185.42-487.07 µg/m^3)].

A study of PM1.0, PM2.5, and PM10 was conducted by Clean Air Commission of Vienna, Austria [19] at three urban [site 1: (PM1.0: 14.9+7.7; PM2.5 18.6+10.7; PM10 26.5+13.3); site 2: (PM1.0: 14.7 +8.5; PM2.5 18.8 +12.0; PM10 29.9+19.0; site 3: (PM1.0: 17.5+10.2; PM2.5 21.1+12.9; PM10 31.0+17.0], and one rural site 1: (PM1.0: 12.4+6.1; PM2.5 15.0+8.6; PM10 21.1+10.5) with observation of no seasonal influence at rural site. The present study focused on urban sites during one season only but higher levels of pollution were seen in traffic intersections under the classified categories (Tables *4* and 5). Contrastingly, the study of two Beijing sites, namely Chegongzhuang and Tsinghua, were assessed [20] for PM2.5 and found ranging between 37 and 357 µg/m^3 and found PM2.5 to be highest in winter and lowest in summer. It is observed in an Australian study [21] that the concentration of particulate matter proportionately decreases for PM2.5 with increasing distance from road reaching to 40% of that level at 150 m distance.

A review report by Central Pollution Control Board, CPCB [22] has noted an average annual exposure level; of PM2.5 to be 34.39, 43.44, and 47 in the years 2000, 2005, 2010, 2011, and 2013, respectively. The present study has noticed PM2.5 vs PM10 (G2: $p=0.03$), PM1.0 vs PM2.5 (G2: $p=0.03$), and VLIs PM2.5 (G1+G2+G3: $p=0.05$).

In the present study, the ratio of DT and NT value assessments for VLI, TVOC, CO_2, HCHO, and PM10 shows higher proportion in DT for select values as per inter group variability [VLI (G1: 1.4; G2: 1.01); TVOC (G1:2.45; G3:3.66); CO_2 (G1: 1.3; G2:1.19; G3:1.18); HCHO (G1: 2.69; G3: 03.00); PM10 (G1: 2.5); Table *2*].

A study conducted in Italy [23] observed PM10 and PM2.5 at six sites of a town to be in the range of 41.5-89.5 µg/m^3 and 34.0-62.5 µg/m^3 from the Advanced Research Project Agencies (ARPA) database. The present study results (Table *3*) similarly show the same as Spearman's correlation coefficient for VLI vs PM2.5 ($p=0.05$), PM2.5 vs PM10 ($p=0.02$), PM1.O vs PM10 ($p=0.00$), and PM1.0 vs PM2.5 ($p=0.01$) like the seasonal trends of particulate matter from heterogeneous traffic near urban roads assessed at IIT Madras [24] to be higher especially for PM10 concentration as per stated norms of World Health Organization (50 µg/m^3) and Indian National Ambient Air Quality Standard (NAAQS; 100 µg/m^3; 2010). The Spearman correlation coefficient (PM10 vs PM2.5; 0.75; PM2.5 vs PM1.0; 0.92, PM2.5-10 vs PM1.0:0.11) was also assessed in a study by National Public Health Institute, Finland [25]. PM2.5 was also found raised as compared to the

World Health Organization (25 µg/m^3) and Indian NAAQS (60 µg/m^3) during three fourth of the time. The post-monsoon season (PM10:189, PM2.5:84, PM1.0:66 µg/m^3, winter season (PM10:1135, PM2.5:73, PM1.59 µg/m^3, summer season (PM10:102, PM2.5:50, PM1.34 µg/m^3). A Netherland-based study [26] concluded black smoke and NO_2 concentration to higher near motorways. A study conducted at the University of Dhaka [3] observed mean PM1.0, PM2.5, and PM10 concentration to be 46.1+13.4, 76.0+16.2, 203.9+44.8 µg/m^3, whereas NO_2 and TVOC were 0.076+0.007 ppm and 90.0+46.0 ppm in even indoor environment.

A sampling at six locations was conducted for Air Quality Indexing in Bangalore city [27] with AQI ranging 42.64-140.52 (unhealthy for the sensitive group), while noting the parameters like temperature, relative humidity, wind speed, and rainfall. In the present study, the ratio of DT and NT value assessments for VLI, TVOC, CO_2, HCHO, and PM10 shows a higher proportion in DT for select values as per intergroup variability [VLI (G1: 1.4; G2: 1.01); TVOC (G1: 2.45; G3: 3.66); CO_2 (G1: 1.3; G2: 1.19; G3: 1.18); HCHO (G1: 2.69; G3: 03.00); PM10 (G1: 2.5); Table *2*].

Another study conducted by Southern California Particle Center and Supersite of Centre for Occupational and Environmental Health, Los Angeles [28] measured temperature (Celsius) [summer: 30.3+3.7; winter 23.2+4.0], relative humidity (%) [summer: 66.4+14.8; winter 43.1+21.4], wind speed (m/s) [summer: 1.36+0.66; winter 1.27+0.67], traffic density at 405 freeway (vehicles/min) [summer:231 +30; winter 236+27], traffic density at 710 freeway (vehicles/min) [summer: 203+12; winter 200+11]. The environmental conditions in the present study noted temperature, dew point, humidity wind pressure, wind speed, UV radiation and visibility also with predominantly high humidity (DT: 81.53%, NT: 86.30%), and low visibility (DT: 5.5 unit; NT: 4.85 units; Table *8*).

Particulars	Temperature (Celsius)	Dew point	Humidity (%)	Wind pressure (Mb)	Wind speed (Km/H)	UV	Visibility (units)
DT	26.75	24.35	81.35	1003	19.7	Low	5.50
NT	25	24.2	86.3	1004	14.2	Low	4.85

TABLE 8: Mean of various environmental parameters during DT and NT at included TIs under the study (n=20)

DT: daytime, NT: nighttime, TI: traffic intersections.

Conclusions

The present study developed a new index namely VLI, which shall be more realistic to be adopted in the future for assessment of vehicular traffic concentration. The assessed increased levels of PM10, TVOC, CO_2, and HCHO at all TIs under study including high, medium, and low traffic areas indicate moderate to severe public health threats to the resident community, commuters, nearby schools, and other people-centric facilities. These may lead to cough, asthma, bronchitis, stroke, and premature death among the exposed population as per their demographic and epidemiological profile. The presence of submicron particles (>0.25 µm, >0.3 µm, >0.5 µm, >10 µm) in almost all sites of traffic intersections in DT and NT indicates public health threats due to deposition of these particles into alveoli leading to irreversible pulmonary damage. Hence, there is a felt need for comprehensive strategic pollution prevention and control policy-based initiatives for primary prevention-based public health interventions in varied geological settings, especially in developing nations.

Additional Information

Disclosures

Human subjects: All authors have confirmed that this study did not involve human participants or tissue. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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Applicability of Native L-Arginase produced by *Streptomyces plicatus* KAR73 as Antineoplastic Agent

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Most of the cancer cells require high quantity of arginine for sustaining their fast-metabolic rates. Limiting supply of arginine to cancer cells using arginase may prove to be of great therapeutic value. The arginase produced by a micro organism isolated from soil has been used in industrial production of ornithine, however its use in anticancer activities is scarcely studied. This study optimized soybean meal supplemented basal media for Arginase production. Arginase was purified using ammonium sulphate precipitation, sephadex G-100 column chromatography and DEAE chromatography achieving 79.2% purification fold and 24.26% yield. It had 23 KD molecular weight as determined using Native PAGE and was active at considerably wide pH range of 6–10 and temperature 30–50°C. Whereas the maximum arginase activity was noticed with Mn^{2+} ions followed by polyvinyl pyrrolidine (PVP) at 70 mM substrate concentration, the maximum inhibition of activity was caused by CuC. *Streptomyces plicatus* KAR 73 produced arginase on mouse mammary cell line (CID 9) was not inhibited by the arginase upto 6.5 U/mL. Significant ($p< 0.001$) inhibition in Mouse mammary tumor (C1271) cell lines was observed with IC_{50} 5.2 U/mL. The ornithine has been produced earlier with *Mycoplasma* and *Clostridium* by other researchers but production of native arginase from *Streptomyces* specifically for anticancer activities has not yet been reported. The present study infers that Arginase produced from native *Streptomyces* has shown promising results thereby enabling feasibility assessment towards cost effective industrial production of arginase.

Keywords: Anticancer, Apoptosis, Arginase, Cytotoxicity, Mammary tumor

Introduction

Cancer, with its high morbidity and mortality, poses serious threat to the sustenance and quality of life. Hence, vigorous, coordinated and evidence-based efforts are needed to prevent and treat carcinomas. It is noticeable that normal cells die during various developmental and stress processes. Since, apoptotic processes remain inactive in cancer cells, effective strategies are required to counter tumor propagation.[1] Normal adult cells synthesize adequate levels of arginine to maintain cellular metabolic functions.[2] It becomes conditionally indispensable during tissue injury, metabolic disorders and disease conditions demanding increased supply of arginine from neighbouring tissues or through diet. Rapidly growing tumor and cancer cells require a nutrient-rich environment to maintain their growth. Arginine serves as an intermediate in the urea cycle as well as a precursor for protein, polyamine, creatine and nitric oxide. Therefore, restricting access to arginine by arginase may cause death of malignant cells resulting in defective cell cycle check-point control.[3] Normal healthy cells, by contrast, become inactive and remain viable under arginine starvation, displaying full recovery upon return to arginine rich conditions.[4] Some arginine hydrolysing enzymes including mycoplasma derived arginine deiminase and human recombinant arginase have been developed for therapeutic purposes.[5]

Arginases (EC 3.5.3.1), also known as arginine amidinase, L-arginase, canavanase and arginine transamidinase, are manganese containing enzymes converting arginine into ornithine and urea. Apart from mammalian tissues, these have also been isolated and characterized from worms, molluscs, fishes, bacteria, fungi, yeast, actinomycetes, algae and plants. Among microorganisms actinomycetes, *Entamoeba histolytica*e, *Plasmodium falciparum*, *Bacillus anthracis*, *Rummeliibacillus pycnus*,

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Agaricus bisporus, *Enterococcus faecalis*, *Evernia prunastri*, *Xanthoria parietina*, *Peltigera canina*, *Fasciola gigantica* and *Squalus acanthias* are reported to produce arginase that differ widely in their molecular structure.[6–7] Use of native arginine deiminase (ADI) for therapeutic purposes has also shown antigenicity.[8] The pegylated form of ADI has shown its strong anticancer activities against HCC and malignant melanoma.[9,10] However, unfavorable effects under *in vivo* conditions remain to be thoroughly explored before clinical application of such conjugated enzyme system.[11]

Precise role of arginine in cancer may include influence tumour initiation, promotion, progression, apoptosis, tumour-cell adhesion, angiogenesis, differentiation, and immunosuppression.[12] The efficiency, effectiveness and applicability of native L-arginase produced by *Streptomyces plicatus* KAR73 as anti-neoplastic agent are illustrated in this paper. In addition, economic feasibility for production of microbial therapeutic enzymes may be accentuated using *Streptomyces plicatus* KAR73. Hence, the objective of this study included production, characterization and anticarcinogenic-potential assessment of native arginase derived from *Streptomyces plicatus* KAR73.

Materials and Methods

Chemicals and Reagents

The chemicals and reagents were procured from Himedia Laboratoties Pvt Ltd, Mumbai, India; Thermo Fisher Scientific India Pvt Ltd, Mumbai, India and used as per manufacturer's instructions. *Streptomyces plicatus* KAR73, an isolate of cultivated field soil of district Bhopal, Madhya Pradesh, India (identified using 16S RNA and biochemical test reported elsewhere) was maintained on International *Streptomyces* Protocol medium-2 (ISP-2). The broad range mol wt marker of 29 KDa, 45 KDa, 66 KDa, 97 KDa, 116 KDa and 200 KDa mol wt were obtained from Sigma Chemical Company. Mouse mammary tumor cell line C-1271 was obtained from Cell line Repository, National Centre for Cell Sciences, Pune, India. The generally used media, buffers and reagents were obtained from HiMedia Pvt. Ltd.

Microorganism

The actinomycetes *Streptomyces plicatus* KAR73 used in the study was islaoted from cultivated field soil of karond area of Bhopal, soil characteristics include black colour with pH 7.8, the strain was selected for further studies after primary screening of 231 strains of Actinomycetes for production of arginase enzyme.[13]

Production of Arginase

Production of L-arginase by *Streptomyces plicatus* KAR73 was done on pre-optimised media containing (g/L) Glucose, 10.0; K_2HPO_4, 0.40; $MgSO_4.7H_2O$, 0.05; NaCl, 0.01; $FeSO_4.7H_2O$, 0.01 supplemented with L-arginine 0.1 (w/v%). Inoculum density equivalent to 0.500 Optical density (2×10^6 Colony forming units/mL) was added to the medium in a ratio of 5:95 (v/v). The variation in pH of medium and incubation temperature was done to assess optimum condition for production of arginase under shake conditions (150 rpm) for a period of eight days. A set of flasks were terminated after every 24 h and whole culture broth was centrifuged at 3,000 × g for 10 min at 4°C. Arginase activity and protein content were measured in supernatant, while pellet was used to measure growth. Growth (biomass) was quantified in terms of dry weight of biomass after overnight drying at 100°C. All the experiments were conducted in triple sets throughout this study.

Assay for Arginase Activity and Protein Quantification

Arginase activity was measured by the modified method of Roman and Ruy's Roman.[14] Reaction mixture contained 20 mM Tris HCl buffer (pH 8.0), 2 mM $MnCl_2$, 100 mM arginine (pH 7.5) and enzyme sample in total volume of 2 mL and incubated at 37 °C for 1 h and stopped by adding ice cold trichioroacetic acid 10% (w/v) followed by centrifugation at 10,000 × g for 10 min at 4°C. Blank was processed in similar manner except that addition of ice cold trichioroacetic acid 10% (w/v) was done prior to addition of enzyme sample. Amount of L-ornithine present in supernatant obtained from above was measured using HPLC Yu.[15] The analysis of ornithine level was performed after filtering the supernatant through 0.45 µm PVDF filter, followed by derivatizing with 6-aminoquioly-N-hydroxy–succinimidyl carbonate. The derivatives were separated with a Nova-Pak$_{TM}$C 18 column. Content of Ornithine was calculated by referring to the standard curve. One unit of enzyme was defined herein as the amount of arginase that produces 1 µmol of ornithine per minute at 37°C. The protein content was also determined in enzyme samples according to the method of Bradford Bradford[16] using Bovine Serum Albumin (Himedia, India).

Purification of Arginase

Ammonium Sulphate Precipitation

Purification of arginase from cell free culture broth was carried out in sequential ascending manner. Cell free culture broth was filtered twice with 0.22 µm millipore filters. The resultant broth was saturated with solid ammonium sulphate with continuous stirring at 4°C. Salt addition was done in an aliquot of 1gm per 100 ml broth until complete dissolution of salt. The saturated solution was centrifuged at 10000 × g for 15 min at 4°C. The pellet containing enzyme was dissolved in minimum amount of 10 mM Tris-HCL buffer (pH 7.5) containing 0.5 mM $MnCl_2$, 0.5 µM arginine and 5% glycerol, stored at −80°C. The precipitate was dialysed thrice using dialysis membrane overnight at 4°C against 0.1 mM Tris-HCL buffer (pH 7.5).

Sephadex G-100 Chromatography

Arginase was purified using Sephadex G-100 (2 × 50 cm) column equiliberated with 20 mM HEPES and 0.5 mM $MnCl_2$. Washing of column was done twice. Dialysed, concentrated protein mixture was applied to column and eluted with same buffer with a flow rate of 1.0 ml per minute.

DEAE Cellulose Chromatography

Active fractions obtained from above were pooled and applied on DEAE-cellulose column (2 × 10 cm) equilibrated with 10 mM Tris-HCL buffer (pH 7.5) containing 10% glycerol. The column was washed twice with buffer and enzyme elution was done using a linear gradient of sodium chloride (0.1–0.5 M) prepared in 10 mM Tris-HCL buffer (pH 7.5). The elute was then dialysed using dialysis membrane at 4°C against 0.01 mM Tris-HCL buffer (pH 7.5). The excess buffer from dialysate was removed by keeping the dialysis membrane containing sample in sucrose bed for 24 h at 4°C.

Polyacrylamide Gel Electrophoresis

Molecular weight was determined on 12% (w/v) SDS gel electrophoresis.[17] The purified enzyme was treated with loading dye containing SDS 1% (w/v) and β-mercaptoethanol and boiled for 2–3 min at 100°C and loaded in wells. Sample staking was done at 4% gel. Resolution was carried out at 110 V. The mol wt was determined using broad range mol wt marker (Sigma Chemical Co.) of 29 KDa – 200 KDa mol wt. The gel was carefully removed after electrophoresis and stained with 0.25% (w/v) Coomassie brilliant blue (R-250) dissolved in 50% (w/v) methanol and 10% (w/v) acetic acid followed by destaining with 10% (w/v) acetic acid. Zymography of purified arginase was also performed with native PAGE.

Characterisation of Arginase Activity

Purified arginase was appropriately diluted before characterization studies. The characterization was performed in sequential manner and the optimum conditions of previous experiment were used for successive characterization studies. Buffers systems used for determining pH dependence of arginase activity were 0.01 M acetate buffer (pH 5), 0.01 M phosphate buffer (pH 6–7), 0.01 M Tris HCl buffer (pH 8–9) and 0.01 M borate buffer (pH 10). Effect of temperature on the arginase activity was studied at 20−50°C. Effect of substrate concentration (10–100 mM) on arginase activity was studied at optimum temperature and pH elucidated from above experiment. Activity of arginase in presence of inhibitors 10 mM SDS, 10 mM EDTA, 10 mM PMSF, 10 mM DMSO, 10 mM DTT, 20% (v/v) Triton X-100 and 20% (v/v) Tween-20 was carried out at herein identified optimum pH and temperature. In addition, the effect of metal ions (Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Fe^{3+}) on enzymatic activity was also determined.

Study of Effect of Purified Arginase on Mouse Mammary Tumor (C-1271) Cell Lines

Preparation of Cell Lines for Cell Cytotoxicity Assay

Mouse mammary tumor cell line C-1271 was obtained from National Centre for Cell Sciences, Pune, India. These were cultured in complete growth medium Dulbecco's Modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum, 0.1m M non-essential amino acids, and 1 mM sodium pyruvate 100 µL/mL, penicillin 100 µL/mL, streptomycin 100 µl/mL and 0.2 mM L-glutamine. These cell lines were maintained at 37°C in humidified atmosphere of 95% air and 5% CO_2. Sub-culturing was performed every 24 h with cells from subconfluenet cultures after treated with Trypsin-EDTA solution (Himedia Pvt. Ltd.). After trypsinization, cells were counted using nebular cell counter and diluted appropriately. The cell viability was checked using trypan blue assay. The 99% viable cells were used for further experimentation.

MTT cell Cytotoxicity Assay

Quantitative cell cytotoxicity assay under different treatment conditions was determined with

3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H- tetrazolium bromide (MTT) assay.[18] Cell density of 1×10^4 cells (ml^{-1}) were plated into 96 well tissue culture plates. The cells were allowed to reach confluence for 24 h at 37°C. The purified arginase was added in increasing concentration of 0.1 to 6.5 U/mL and incubated for 24 h. Subsequently, cytotoxicity was determined by MTT Assay using EZ count™ MTT Cell Assay Kit. The optical density was measured at 570 nm (ELISA reader, Multiskan Ascent, Thermo Scientific, India). The half maximal inhibitory concentration (IC_{50} value) was defined as 50% decrease in cell viability.

Trypan Blue Assay

Cells with density 10^4 cells/mL were seeded on plates and were allowed to multiply and adhere for 24 h at 37°C and 5% CO_2 in a humidified atmosphere. Successive sequential concentration of Arginase was added to all the cell lines C-1271 in different Sets once they reached confluency. After 1 h of treatment, cells were trypsinized and to appropriate cell density of cell line (s) Trypan blue dye was mixed and incubated for 1 min.[19] Thereafter, 10 µl of this cell-dye suspension was loaded in Neubauer chamber and observed under low (100x) and high (400x) power of an inverted microscope. Number of viable (bright cells) and non-viable cells (stained blue) were counted.

Colony Forming Unit Assay Using Crystal Violet Staining

Cell density (10^4 cells/ml) was seeded on plates and allowed to adhere and multiply for 24–48 hours at 37°C and 5% (v/v) CO_2 in a humidified atmosphere. The used up medium was discarded and fresh medium was added to each plate. Arginase enzyme was added as mentioned before; a set of plates untreated with arginase was labeled as control. On completion of desired incubation period plates were washed twice with Phosphate Buffer Saline followed by fixing with 10% formalin for 15 minutes. Staining was done with Crystal Violet for 10 minutes,[20,21] Excess stain was removed by washing with Phosphate Buffer Saline (pH 7.2). Colony forming units were counted using stereo and compound microscope.

Apoptosis Assay using Annexin V- FITC and PI Assay

The cells were cultured in tissue culture plates and treated with arginase, after 6 hours and 24 hours of incubation number of viable cells, apoptotic and necrotic cells were analysed.[18] Briefly, the cells after treatment with purified arginase were washed with PBS (pH 7.2) and then trypsinized with Trypsin-Ethylene diamine tetraacetic acid (EDTA) solution for 1 minute. These cells were thereafter collected and mixed with Dulbecco's Modified Eagle's medium (DMEM) at 1600 rpm. The pellet was washed and stained with Annexin V-FITC antibody and PI. The cells were scanned for fluorescence intensity using Flow Cytometer (Lab Quanta). The control was also processed in same manner except that it was not treated with arginase.

Statistical Analysis

Statistical analysis was done using Epi Info™ Software available online from Division of Health Informatics & Surveillance (DHIS), Center for Surveillance, Epidemiology & Laboratory Services (CSELS), Centers for Disease Control and Prevention (CDC). The analysis was done for experimental and control groups. Graphical representations of the data were performed using GraphPad Prism.

Results and Discussion

Production Media for Arginase

Streptomyces plicatus KAR73 was found to produce extra-cellular arginase with 450.5 U/mL activity and 3.821 mg/mL protein (Table 1). The production of arginase by *Streptomyces plicatus* KAR73 is found in all temperature and pH range tested. The overall peak effectiveness is best amongst three different temperature constants (28°C, 37°C and 42°C) at pH range of 5–10. The most suited one with best results is observed at pH 7.0.

Optimization of Temperature and pH Tolerance Favor Production Potential

Across temperature range of 28–48°C, there is homogeneity in peak observance for production of arginase to be highest at pH 7 both for individual

Table 1 — Purification of L-arginase produced by *Streptomyces plicatus* KAR73

Purification steps	Volume (ml)	Enzyme activity (U/mL)	Total units	Protein content (mg/ml)	Specific activity	Recovery percentage (%)	Purification fold
Culture filtrate	100 ml	450.5	45050	3.821	117.90	100	—
Precipitated by ammonium sulphate	17 ml	1974.84	33572.38	2.209	894	74.52	7.58
DEAE-Cellulose chromatography	14 ml	2134.8	29887.2	1.15	1856.34	66.34	15.74
Sephadex G-100	7.8 ml	4306.43	32728.86	0.832	5176	72.65	43.90

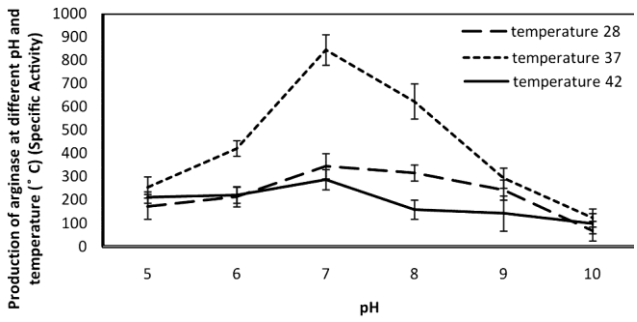
temperature constant and variation in the temperatures for the same pH. This implies three fold increases in production of enzyme at temperature 37°C as compared to temperature 28°C (Fig. 1). Similar results have been observed with *Alcaligenes faecalis*.[22] The extracellular production of arginase was pH dependent at 37°C which might be due to alteration in permeability of membrane.

Purification of Arginase

Complete recovery of protein from culture broth of *Streptomyces plicatus* KAR73 was achieved on 65% (w/v) ammonium sulphate saturation. The purification fold increased three times (15.74 to 43.90) when the purification step was raised from DEAE- cellulose chromatography to Sephadex G-100 with respective yields of 66.34% and 72.65% (Table 1). It is hence inferred here that purification upto Sephadex G-100 level may be commercially useful. It is noteworthy that the completion of designated steps of purification provides better results including gain in protein contents and purification fold as depicted by highest specific gravity in DEAE- cellulose chromatography.

Molecular Weight of Arginase

Studies revealed that *Streptomyces plicatus* KAR73 produced only one type of arginase with its molecular weight being 23 KDa. While ensuring purity of arginase by SDS PAGE, the zymography has clearly indicated enzymatic action with noticeable impact. Purified arginase of molecular weight 75 KDa, has been isolated and studied from *Pseudomonas aeruginosa* IH2.(18) The molecular weight (23 KDa) of derived arginase from *Streptomyces plicatus* KAR73 in this study is comparable with that of human Arg 1 having molecular weight in the range of 35–105 KDa.[23,24] Metabolic and genetic diversity of *Streptomyces* sp. is vast and require extensive studies



Fig, 1 — Effect of pH and temperature on production of Arginase by *Streptomyces plicatus* KAR-73 grown on medium containing glucose and arginine as carbon and nitrogen source under shake conditions (150 rpm)

to completely define them. Also, limited availability of the relevant published literature on *Streptomyces plicatus* produced arginase is although not self speaking about the similarity of size of arginase obtained herein with that of human arginase but the experimental study revealed it to be in same range. This range is further verified by a study from Warszawa, Poland observing human heart arginase to be 30 KDa[25] and Arginase II from *Heteropneustes fossilis* showing 96 KDa band on native PAGE in another study from Varanasi, India.[26]

Characterization of Arginase Activity

The arginase was found to be active in broad range of pH from 6 to 10 with optimum activity at pH 7.0–8.0 (Relative activity 99.14%–100%) (Fig. 2a). The range of pH 6.0 to pH 10.0 observed in our study on activity of purified arginase produced is in resonance with the cellular environmental pH and hence anticarcinoma potential has wide ranging applicability in on site anticarcinoma action. The maximum activity of arginase produced by *Streptomyces plicatus* KAR 73 as pH (7.5 pH) in this study is in near absolute resonance with human physiological pH (7.2–7.4 pH), whereas other studies show maximal activity in outlier pH viz., *Bacillus anthracis* arginase activity occurred with nickel at an alkaline pH 9.0 in a study from University of South Alabama, USA.[27] Also, it is well known fact that cancerous cell have slightly acidic pH and thus the arginase obtained from *Streptomyces plicatus* KAR 73 may prove to be more suitable for cancer treatment as compared to those obtained from Buffalo liver which was optimally active at pH 9.2.(28) Wheatley and his co-workers[5] have reported that bovine liver arginase is remarkably heat resistant enzyme with a long life on storage at 4°C in lyophilized form and is active at pH 7.2 than at pH 9.9 while arginase derived herein was maximally active at pH 7.5.

Arginase from *Streptomyces plicatus* KAR 73 was active and stable at 25−42°C with 100% relative activity at 37°C (Fig. 2b). It is noteworthy that relative activity of arginase was almost stable in the temperature range 30–37°C with progressive difference of 0.002%. Cai and coworkers[8] have reported production of thermostable arginine deiminase by *Enterococcus faecalis* SK23.001. Thermostable arginase from *Geobacillus thermodentrificans* NG80-2 was found to be optimally active at pH 9 and temperature of 80°C.[21] In the present study, the purified arginase from

Streptomyces plicatus KAR 73 is found active in a broad range of temperature and tolerated even 50°C. The purified arginase was found to be active at temperature range of 30–40°C thereby making it suitable for therapeutic purposes under physiological conditions. Recently, purification of arginase from Camel liver done using heat denaturation, ammonium sulphate precipitation, DEAE-cellulose, SP-Sepharose and Sephadex G 100–120 chromatography columns has shown its optimum temperature activity at 70°C.[29] The maximum arginase activity was noticed at 70 mM Arginine concentration (Fig. 2c).

All the inhibitors and detergents used in the study caused reduction in enzyme activity, EDTA caused 59.7% inhibition and PMSF caused 21.8% inhibition, respectively. Arginase activity was found to be even higher than that of control in presence of Mn^{2+}, while only 15.7% residual activity was observed with Cu^{2+} metal ion as compared to control (Fig. 2d). Arginase activity inhibition was noted with other metal ions as well. Interestingly, presence of PVP did not influence arginase activity under *in vitro* conditions. The observed inhibition of arginase by CuC, FC and CaC may assist in the controlled production, activity and interaction of arginase with concurrent use of drugs, chemicals and other therapeutic interventions. In this study, enhancement in the activity of arginase was observed in presence of Mn^{2+} and PVP. It is noticeable that Arginases Arg I and Arg II from *Fasciola gigantica* have also been observed activated by Mn^{2+} and inhibited by Fe^{2+}, Ca^{2+}, Hg^{2+}, Ni^{2+}, Co^{2+} and Mg^{2+} ions.[30] Similarly, recombinant *Plasmodium falciparum* arginase activity has been found to be dependent on Manganese.[31] D-arginase from *Arthrobacter sp* KUJ8602 revealed an optimum pH of 9.5 and requirement of Zn^{2+} for activation instead of manganese ions.[31] Activity of *Saccharomyces cerevisiae* arginase was found to be dependent on Mn^{2+} ions, and removal of ions via dialysis caused significant changes in its spectra.[32]

Lytic Potential Confirmed by Early Apoptosis, Late Apoptosis and Dead Cells

The Mouse mammary tumor cell line (C1271) was found to be inhibited by different concentrations of arginase (0.1 to 6.5 U/mL) with IC_{50} 5.2 U/mL (Fig. 3). Dead cells were confirmed post staining and treatment with purified arginase under visualization through Trypan Blue staining of C1271. The disseminated fragmentation of CFU assay, as

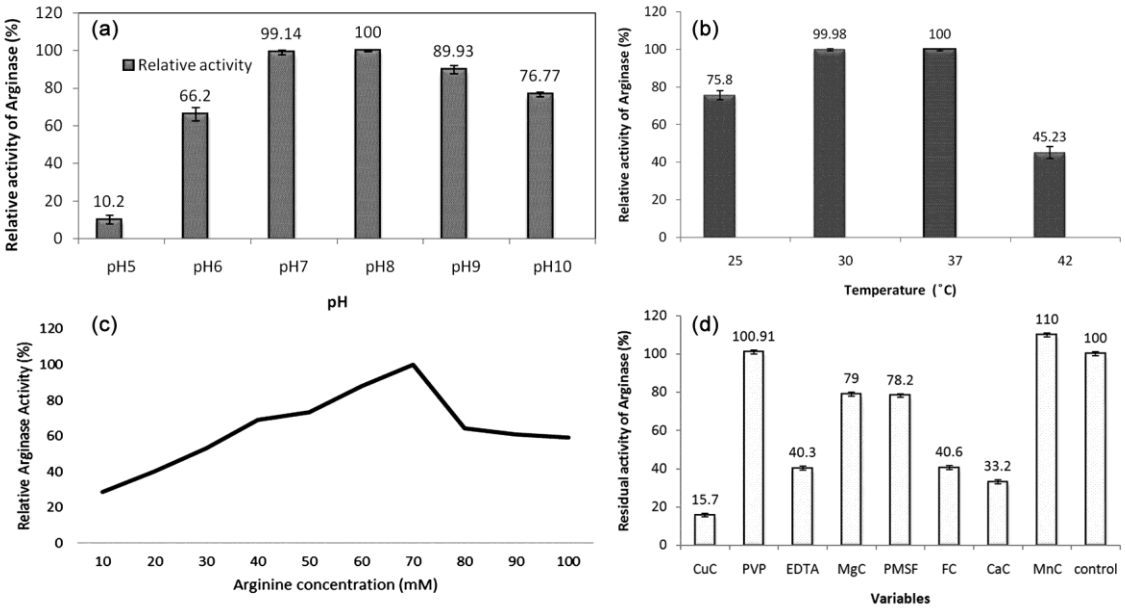


Fig. 2(a–d) — (a) Relative activity of purified arginase produced by *Streptomyces plicatus* KAR 73 at different pH under *in vitro* conditions; Assay conditions temperature 30°C and buffers (pH 5–10), (b) Relative activity of purified arginase produced by *Streptomyces plicatus* KAR 73 at different temperature under *in vitro* conditions; Assay conditions pH 7.5 and temperature (25°C–42°C). **Copper chloride (CuC), PVP, EDTA, magnesium chloride (MgC), PMSF, ferric chloride (FC), calcium chloride (CaC) and manganese chloride (MnC)*, (c) Effect of substrate concentration on arginase activity; The arginase activity is expressed as relative activity the assay conditions were pH 7.5 and 37°C temperature and (d) Effect of activators and inhibitors on arginase activity; The results are expressed in terms of residual activity remaining after treatment with activators and inhibitors under in vitro conditions; Assay conditions pH 7.5 and temperature (37°C)

observed through crystal violet staining of arginase treated plate in comparison to same staining for control plate illustrates the non-viability of cells including clumps of dead cells (Fig. 4). The confirmation was met by Annexin V FITC staining showing live apoptotic and dead cells through morphological visualization. The observance of 90.44%, 3.63%, 5.26% and 0.68% being apoptotic, early apoptotic, dead cells, and viable cells respectively, underlines that there is high lytic potential of arginase for carcinoma cells (Fig. 5).

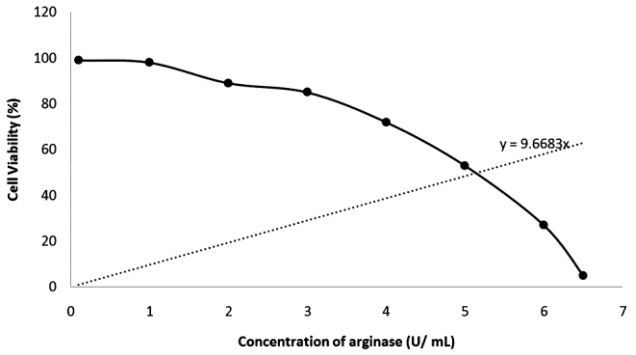


Fig. 3 — IC_{50} of Arginase against C1271 cell lines determined using MTT assay

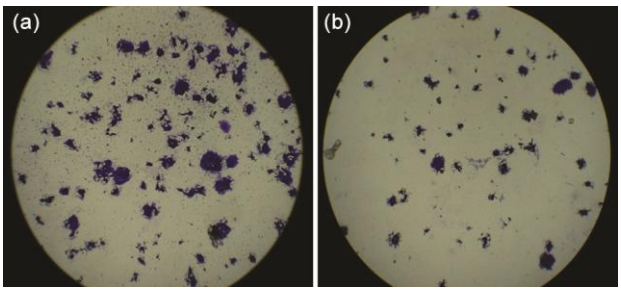


Fig. 4 — Colony forming unit assay: C1271 cell line were cultured in complete growth medium DMEM with 5% FBS, 24 h incubation period (a) Microscopic observation of control plate (first quadrant) after Crystal violet staining; (b) Microscopic observation of arginase treated plate (first quadrant) after Crystal violet staining

Arginine deiminase (ADI) has been reported to exert inhibitory effects on cancer cells under *in vitro* and *in vivo* conditions as depicted in earlier studies for Pegylated enzymes.[11] It is further approved by the present study even for native enzyme(s). The result of cell cytotoxicity assay (Fig. 5) illustrates the acceptability of arginase for use in the human tissues with unhindered and un-interfered biological activity required for normal cell functioning and maintenance of homeostasis of body systems.

It is evidenced by apoptosis and cell death even in the presence of Arginosuccinate synthetase enzyme in this study, whereas it was hypothesized till now that its absence is essential for any effective internvention.[12,23,24] Resonance of observations of this study with natural occurrences in cancer cell growth and development is found unique for cellular milieu. It therefore makes the arginase borne of *Streptomyces plicatus* most suited for effective interventions throughout the spectrum of cancers and their stages. However, the ADI has two major disadvantages. Firstly, ADI is not produced by mammals and must be derived from microbes. As a consequence, nascent ADI is strongly antigenic in mammals.[33] Secondly, ADI has a short circulating half-life in mammals (approx. 5 h) and is to be essentially administered in large daily dose to inhibit tumors. The ADI has also been formulated with polyethylene glycol to produce ADI-SS PEG 20,000 MW, which is found safe and non toxic in mice.[11] It has also been stated to be arginine specific and has no role for other

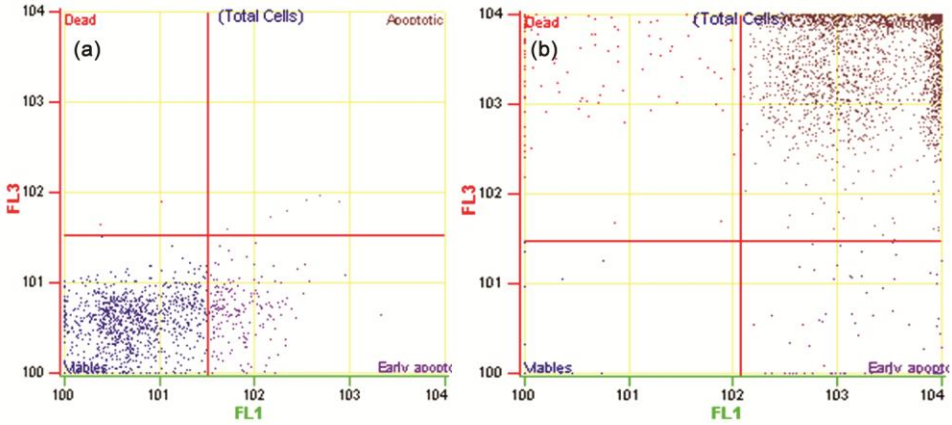


Fig. 5 — Dose and time dependent cycle of early apoptosis- apoptosis- death among C1721 mammary carcinoma cells using purified arginase derived from *S. plicatus* KAR 73; Flow cytometric analysis was conducted using Annexin V-FITC/PI for (a) After 1 h showing number of viable cell, early apoptotic and apoptotic cells (b) After 24 h showing quantum of early apoptotic, apoptotic and dead cell

Arginosuccinate synthase thereby proving anticancer activity of arginase derived from *S. plicatus* KAR 73 in this study.

Conclusions

The arginase derived from *Streptomyces plicatus* KAR 73, as illustrated vide this study, is facilitatory to anticancer research and its industrial applications. The arginase produced herein is within the size range of human arginase thus assuring it to be bioacceptable with special reference to antigenicity. Another highlight of this study is physiologically ambient similarity of *Streptomyces* sp. borne arginase with human arginase as urea and ornithine are byproduct of both these unlike commercially available pegylated arginine deiminase having outcome as citrulline and ammonia. It is hence concluded that *in vitro* effect of arginine deprivation on adenocarcinoma cell lines through native arginase is a promising way forward to the carcinoma therapy. The ray of hope for the public health based utility of arginase in cancer treatment is indeed bright since autophagy targeting drugs in combination with standard chemotherapies will add to the multipronged approach for cancer therapy.

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Review

COVID19 associated mucormycosis: Is GRP78 a possible link?

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ABSTRACT

This review aimed to study molecular mechanisms for high incidence of life-threatening mucormycosis infection in COVID19 cases during second wave of SARS CoV2 pandemic in India. Hyperglycaemia, impaired immunity, acidosis, raised ferritin, glucocorticoid therapy, and COVID19 specific other factors have been implicated in pathogenesis of COVID19 associated mucormycosis (CAMM). Endoplasmic reticulum chaperone 'Glucose Related Protein 78' (GRP78), also involved in SARS CoV2 entry, is the host receptor for invasion by Mucorales. GRP78 is over-expressed by SARS CoV2, hyperglycaemia and ferritin. Delta variant of SARS CoV2 and indiscriminate use of steroids were distinguishing features of second wave and appear to upregulate GRP78 through intricate interplay between internal and external milieu. Common invasive fungal infections like candidiasis and aspergillosis, not utilizing GRP78 as receptor, were inconspicuous. Further molecular research to unravel mechanisms involved in the pathogenesis of CAMM shall effectively complement existing strategies for its prevention and treatment.

Contents

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Introduction

The Severe Acute Respiratory Syndrome Corona Virus 2 (SARS CoV2) causing Corona Virus Disease (COVID19) has affected more than 30 million people and caused 433,589 deaths in India [1]. A second surge in COVID19 cases was witnessed in India almost 12 months after the declaration of SARS CoV2 pandemic. Delta variant, which was designated as strain of concern by Centre for Disease Control and Prevention (CDC) for its increased transmissibility and the severity of disease it causes [2], was the dominant strain [3]. An increased incidence of mucormycosis was observed in COVID19 patients during this second wave [4,5]. Mucormycosis being a rapidly spreading fungal infection with life-threatening consequences created concern among medical fraternity and public. Secondary infections with bacteria and fungi were not a common finding in COVID19 cases earlier. Studies reported very low incidences of bacterial co- infections and extremely low rates of fungal co-infections in COVID19 cases as compared to co-infections in influenza disease [6–8]. Similarly co-infections in MERS and SARS CoV1 were also uncommon [6–9]. The fungal co-infections in COVID19 cases reported from other countries were mainly of Aspergillus and Candida infections [10–13]. A review article on cases reports and case series published between December 2020 and April 2021 found 43 cases of mucormycosis in COVID19 cases of which 71% were from India [14]. The aim of the present review was to understand the mechanisms underlying mucormycosis co-infection in COVID19 cases and to explore further avenues for their prevention and treatment.

Method

Literature search for this narrative review was conducted on PubMed for articles published between the years 1980 and August 2021 and on google search-engine for current COVID19 information. The key words used included 'COVID19', 'mucormycosis', 'GRP78', 'heat shock proteins', 'invasive fungal diseases' and 'delta variant'. Titles and abstracts including 'pathogenesis of mucormycosis', 'immune mechanism against mucormycosis', 'invasive fungal infection', 'fungal infections in COVID19', 'haematological findings in COVID19', 'COVID19 associated mucormycosis', 'trends in second wave' and 'GRP78 in COVID19' were identified and full papers were studied.

Mucormycosis

Mucormycosis or zygomycosis is a rare, aggressive, rapidly spreading, angio-invasive fungal infection [15]. This life-threatening infection is most frequently caused by *Rhizopus oryzae* (syn. Rhizopus arrhizus), a filamentous fungus belonging to the family Mucoraceae of the order Mucorales (Fig. 1). Its spores are ubiquitously present in soil, air, and in decaying fruits and vegetables. Unlike other filamentous fungi that are largely opportunistic infections in immunosuppressed hosts like cancer patients and organ recipients, mucormycosis can also affect those with no apparent immune impairment [16]. Mucormycosis has however been associated mainly with diabetes mellitus especially in those with diabetic ketoacidosis (DKA), haematological malignancies, organ transplant, immunocompromise, trauma and neutropenia [17,18]. The fungal hyphae erode the tissues and blood vessels causing endothelial damage, thrombosis and tissue necrosis [19]. Case fatality is high and disfigurement occurs in survivors due to debridement surgeries needed for treatment. Necrosis gives blackish appearance to the tissues. Hence, the term 'black fungal disease' is used for mucormycosis, although the fungus itself is not black.

Epidemiology of mucormycosis

The computational model-based method estimated the prevalence of mucormycosis of 0.14 cases per 1000 population in India [20], whereas the global prevalence is 0.02–9.5 cases (with median of 0.2 cases) per 100,000 population. Thus, the estimated prevalence of mucormycosis in India is 70 times higher than global data [21]. In India, the most common presentation is rhino-orbito-cerebral mucormycosis followed by pulmonary and cutaneous types [22,23] and diabetes mellitus is the most common risk factor.

There has been definitive increase in the incidence of COVID19 associated mucormycosis (CAMM) in India during the second wave. Although recently published scientific data was not available, this unequivocal rise was observed both locally and in other parts of country and was supported by the facts viz. setting up of special medical wards for mucormycosis patients, increase in the number of debridement surgeries, rising demands for antifungal drug amphotericin and declaration of mucormycosis as a notifiable disease in India on 20th May 2021. By the end of June 2021, 40,854 cases of mucormycosis were notified. [24] Such high incidence in

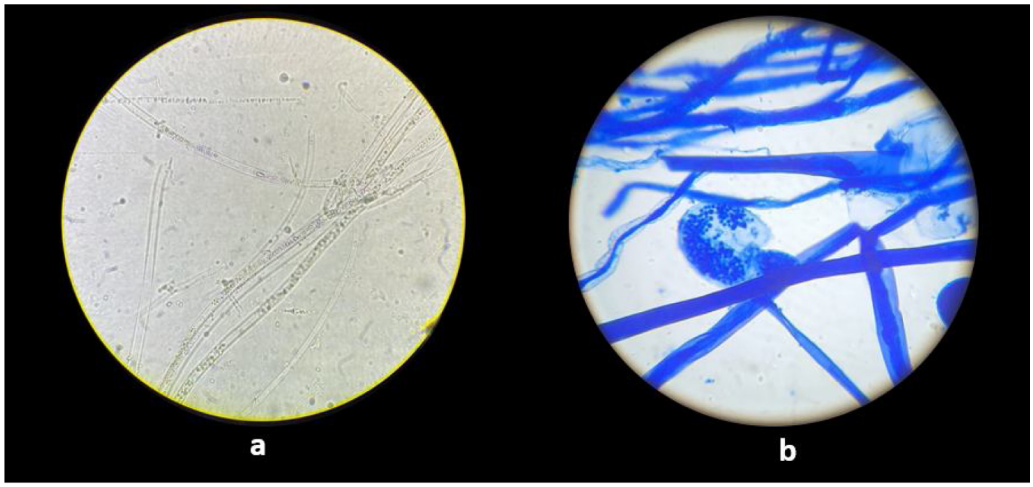

Fig. 1. Microscopic view of Mucorales: (a) KOH examination showing broad, hyaline, aseptate, right angle branched fungal hyphae; (b) LPCB mount showing broad aseptate hyphae with long sporangiophore and terminal round sporangium.

a short span of time created an alarm and concern amongst health authorities.

Potential factors causing COVID19 associated mucormycosis

It is well documented that hyperglycaemia, acidosis and compromised immunity are favourable factors for mucormycosis infection, and iron enhances growth and survival of Mucorales in human host [16,18,19]. In the studies published on CAMM so far, most cases reported were diabetics and most received steroid [4,25–27]. High blood sugar in these cases may have occurred as a result of loss of sugar control in diabetics as a consequence of inadequate availability of routine medical services due to various restriction during the pandemic and inadvertent glucocorticoid treatment. Although steroids had generally been reserved for moderate and severe cases of COVID19 to suppress unwanted host immune responses and cytokine storm, injudicious use among mild and home isolated cases was also in practice. Use of steroids in mild COVID19 cases must have been a consequence of indiscriminate prescription and self-treatment by patients due to mis-information.

It is however worth scrutiny that although glucocorticoids have been used in much higher doses and for longer durations in various medical conditions, mucormycosis has not been a menace in these cases. Also, diabetes mellitus is common in India and uncontrolled diabetes is not uncommon [28]. Hence, although diabetes mellitus and steroids associated hyperglycaemia hold the centre stage as per current knowledge, a deeper understanding beyond this is mandatory in the light of above facts.

There are many factors peculiar to COVID19 that may be considered contributory to CAMM. It has been in scientific discussion that unclean water in humidifiers, bad hygiene of oxygen mask and its tubing and use of industrial oxygen could be the sources of Mucorales. However, local outbreaks of mucormycosis in admitted patients receiving oxygen therapy have not been reported from any medical facility. Use of protective face masks is another unique feature of this pandemic. Repetitive use of the same mask for prolonged duration has been suspected as another source of the fungus as many pathogens can grow due to the moisture trapped in the mask. Yet another probable source could be unclean beddings, unclean vicinity and poor hygiene due to compromised bed side patient care and hospitality accentuated by the fear of contracting COVID19 amongst caregivers. Swab collection for PCR testing with non-sterile and unhygienic nasopharyngeal swabs could be another possibility.

A breach in the integrity of the nasal mucosa due to (a) overenthusiastic use of home remedies like steam inhalation, (b) nasal instillation of substances like oils and lemon juice, and (c) injury from swab collection needs to be investigated as a possible route of entry. Rampant use of broad-spectrum antibiotics, which suppresses normal commensals and reduces bacterial competition on the surface, is another factor.

Zinc, commonly prescribed for its antiviral properties in Covid19, is also an essential micronutrient for fungal growth. The new SARS CoV2 mutant B.1.617.2 or the delta variant, which was the dominant strain during the second wave [29], is another probable factor. Higher viral load has been reported in these cases on RTPCR testing [30]. New variant and high viral loads need to be considered as an important distinguishing factor between this and previous wave. Various in-vitro studies, with dissimilar methodology, reported conflicting effects of glucocorticoid on viral replication. A study on effect of glucocorticoid treatment on respiratory tract cells found increased viral replication, altered inflammatory cell profiles and paradoxical increase of pro-inflammatory cytokines [31]. Elevated acute phase reactant ferritin in COVID19 cases needs to be investigated for being an important source of iron, which is essential nutrient for fungal growth and survival.

However, CAMM was also reported in patients who were not hospitalized, not given steroids, non-diabetic and treated at home with home remedies like steam inhalation, gargling and herbal substances [32], with or without the usual approved treatment regimens. Hence, there may be multifactorial causation and complex interplay therein, enabling the fungus to prosper when the host environment became conducive (Fig. 2).

Evasion of host defences by Mucorales

Two steps are required for Mucorales to infect the host, first is to germinate and second is to invade the tissues. Ability of spores to germinate and form hyphae is the most critical step [17]. Animal studies have shown that the inhalation of Mucorale sporangiospores in immunocompetent animals does not produce mucormycosis [33]. The macrophages suppress spore germination and neutrophils kill the hyphae by oxidative burst in immunocompetent host [34]. The neutrophils start expressing Toll-Like-Receptors 2 (TLR2) on exposure to hyphae and phagocytose the fungus.

The type of immunosuppression determines the susceptibility to and virulence of the fungal infection [35]. 70–100% of the patients with haematological malignancies developing mucormycosis, have been reported to have neutropenia [36]. In diabetic ketoacidosis, there is dysfunctional phagocytosis, impaired chemotaxis and defective intracellular killing of fungus [37]. In a study of 658 COVID19 cases, ketoacidosis was noted in 5 cases and ketosis was present in 42 cases, of which only 15 were diabetics [38]. Thus the presence of ketosis and ketoacidosis in non-diabetics with COVID19 is a possibility. Experimental studies have shown that exposure to corticosteroids renders the alveolar macrophages incapable of preventing germination of spores and ketoacidotic environment prevents the cytotoxic action of macrophages [39]. High ferritin levels, as an acute phase reactant have been found in COVID19 [40,41] and ferritin associated iron induces neutrophil dysfunction [42].

Platelets have also been observed to inhibit fungal germination and hyphal growth, and induce hyphal damage in in-vitro studies [34,43]. Lymphopenia has been associated with disease severity in COVID19 and has also been a frequent finding in cases of mucormycosis with COVID19 [44,45]. T-Lymphocytes play an important role in regulating functions of other immune cells. Lymphopenia, thrombocytopenia and morphologically abnormal neutrophils have been observed in COVID19 cases [46] and any of them, alone or in combination may predispose to mucormycosis. It is however worth noticing that AIDS patients, who predominantly have lymphopenia, suffer from many opportunistic infections but mucormycosis has not been a concern among them. Dysfunctional phagocytosis by neutrophil and macrophage is next critical step for the Mucorales to escape destruction and germinate to form hyphae for host invasion.

In immunocompromised hosts, the main invasive fungal infections are Candidiasis, Aspergillosis, Mucormycosis and Cryptococcosis [35]. The unanswered pertinent observation herein is the extremely rare occurrence of other opportunistic infections usually expected in immunocompromised cases and the selective presence of mucormycosis in some COVID19 cases. Once the fungus germinates and escapes phagocytosis, damage of, and penetration through the endothelial cells or the extracellular matrix proteins lining the blood vessels is the final step for the fungal invasion [17].

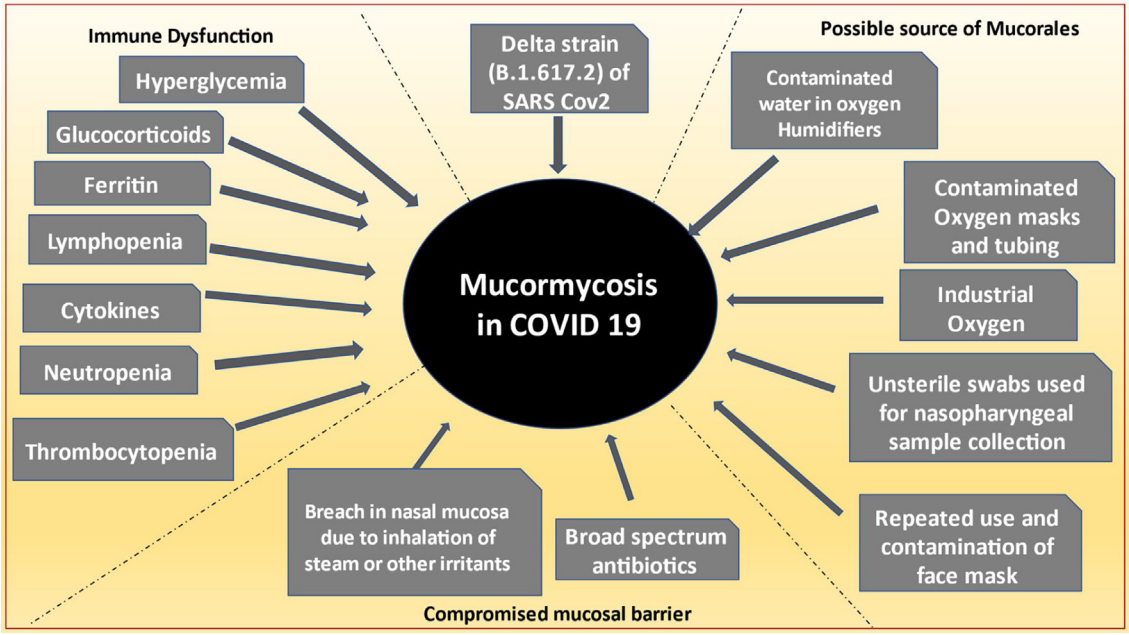

Fig. 2. Causal relevance between COVID19 and mucormycosis.

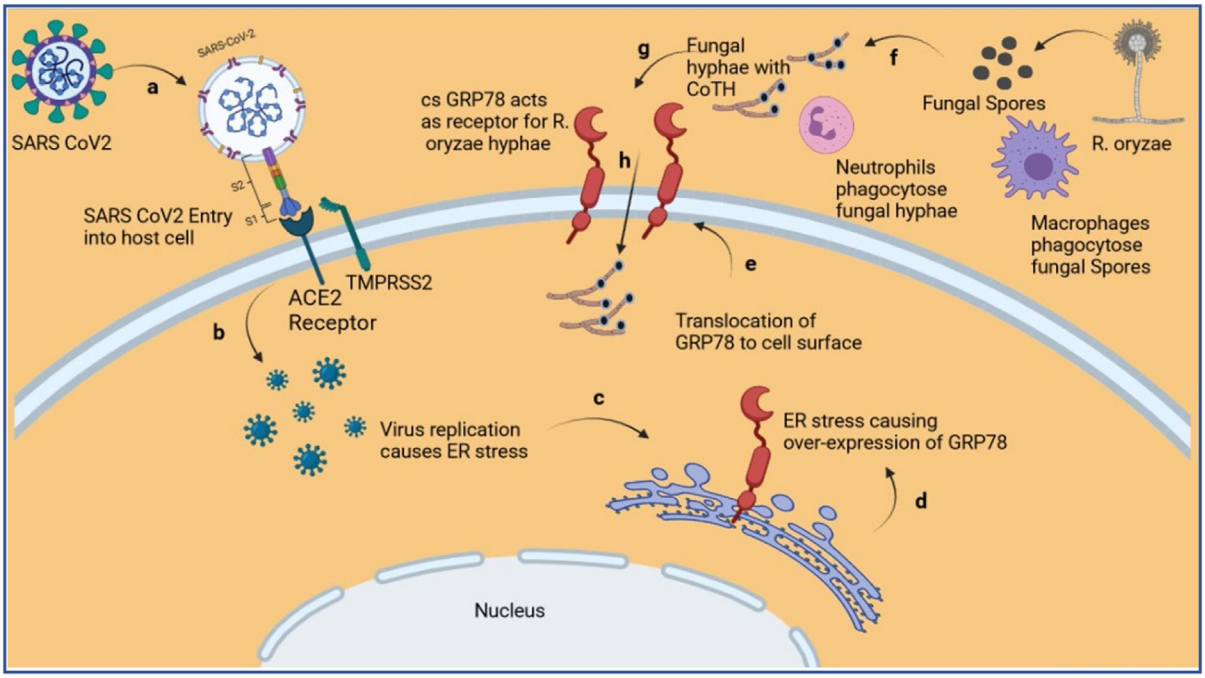

Fig. 3. Diagram depicting over-expression and translocation of GRP78 to cell surface due to SARS CoV2 replication induced ER stress, and invasion of hyphae of *R. oryzae* using cs-GRP78 as its receptor. (a) Entry of SARS CoV2 into host utilizing ACE2 and TMPRSS2 as receptors. (b) Replication of SARS CoV2 inside host cell. (c) ER stress induced due to virus replication. (d) Over expression of GRP78. (e) Translocation of GRP78 to the cell surface. (f) Germination of *R. oryzae* spores into hyphae after escaping phagocytosis by macrophages. (g) *R. oryzae* hyphae escape phagocytosis by neutrophils and utilize cs-GRP78 as receptors, (h) to invade host cell. [18,34,39,42,48–50,55,58] (ACE2-Converting Enzyme type 2; CoTH-Spore coat protein; cs-GRP78-cell surface GRP78; ER-endoplasmic reticulum; GRP78- Glucose Regulated Protein 78; R. oryzae-Rhizopus oryzae; SARS CoV2-Severe Acute Respiratory Syndrome Coronavirus 2; TMPRSS2-transmembrane protease, serine 2) [this figure was created with BioRender.com].

GRP78 as receptor for invasion of Mucorales

The aggressive nature of mucormycosis is due to invasive property of fungal hyphae. Mucorales utilize host Glucose Regulated Protein 78 (GRP78) as receptor for invasion into tissues and endothelial cells [18]. GRP78, also known as Binding Immunoglobulin Protein (BiP) or Heat Shock Protein A5 (HSPA5), is a chaperone protein found mainly in endoplasmic reticulum (ER) and is concerned with folding, assembly and secretion of proteins. GRP78 is upregulated during ER stress and serves as an ER stress sensor. In addition to ER, it is also found on cell surface and extracellular environment [47]. The Spore Coat Protein Homologs CoTH3 and CoTH2 of the fungal hyphae act as ligands for cell surface GRP78 (csGRP78) (Fig. 3). CoTH3 and CoTH2 are widely present in Mucorales and absent in non-invasive fungi [48–50]. Diabetic Keto Acidosis has been found to upregulate CoTH3 and nasal GRP78, which explains

Table 1
The fungal proteins and host receptors in common invasive fungal infections.

Invasive fungi	Fungal protein mediating invasion	Host receptors mediating invasion
Mucorales *	CoTH3 & CoTH2	GRP78
Aspergillus#	Thaumatin-like protein CalA	Integrins
Candida ^	Agglutinin-like sequence (Als)	Cadherins

[* 47–49, # 52, ^ 53].

the rhino-orbito-cerebral presentation of mucormycosis in these cases [51]. The fact that more common invasive fungi viz. *Candida albicans* and *Aspergillus fumigatus* do not utilize GRP78 for invasion needs special emphasis [18] (Table 1). Scope for both targeted anti-GRP78 and anti-CoTH drugs to treat mucormycosis is obvious [48,49].

It has been observed that iron and glucose both enhance the susceptibility of endothelial cells to *R. oryzae* invasion and damage by inducing overexpression of GRP78, wherein the effect of iron was more drastic [18]. Acidosis causes release of free iron from iron binding proteins [18]. As ketosis, ketoacidosis [38] and high levels of ferritin were found in COVID19 cases [40,41] and since iron is essential for the fungal growth, survival and virulence [54], the role of ferritin in CAMM needs to be further investigated.

SARS COV2 and GRP78

The SARS CoV2 spike protein has two subunits S1 and S2. The S1 subunit binds with the host ACE2 (Angiotensin converting enzyme Type 2) receptors and S2 subunit is implicated in fusion of the virus with the host cells. The process of internalization of virus utilizes cathepsin, transmembrane protease, serine 2 (TMPRSS2) and human airway trypsin like proteases (HAT) as well as ACE2 [55], Several studies have shown the evidence of alternative receptors and cofactors that help in virus entry and fusion [56–58], Docking studies have shown interaction between receptor binding domain (RBD) of SARS CoV2 spike protein and GRP78 [56].

The csGRP78 is known to play a role in the infection of host cell by several viruses viz. MERS CoV, Ebola, Japanese encephalitis and Dengue [58]. In fact, GRP78 acts as an important chaperone required for life cycle of all mammalian viruses [59]. Viral glycoproteins of many viruses including SARS CoV2 are the main triggers for endoplasmic reticulum stress. They induce ER stress by accumulation of unfolded protein in the ER lumen and activate Unfolded Protein Response (UPR). The UPR signalling pathways cause upregulation of GRP78 synthesis to handle the unfolded and misfolded proteins. In this process GRP78 is exported out of ER and expressed on the cell surface. GRP78 was four times higher in SARS CoV2 positive pneumonia than SARS CoV2 negative pneumonia [58]. Significant elevation of GRP78 was observed in both SARS CoV2 positive and negative pneumonia as compared to controls but it is still higher in SARS CoV2 positive pneumonia [55].

The increased GRP78 expression on cell surface may further enhance viral entry by positive feedback cycle. Co-localization has been observed between endogenous GRP78 and ACE2 in perinuclear region typical of ER and also on the cell surface. GRP78 may be important for ACE2 trafficking, localization and stability on cell surface, as GRP78 knockdown by siRNA (Small interfering Ribonucleic acid) reduces the level of cell surface ACE2 in parallel with decrease in csGRP78 [57]. Co-localization of GRP78 and SARS CoV2 has also been established in live viral infection. AR-12 (2-amino-*N*-[4-[5-(2 phenanthrenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl] phenyl]-acetamide) a derivative of celecoxib and an inhibitor of chaperone GRP78 (along with many other chaperones) reduces the expression of cell surface ACE2 and GRP78 as well as total GRP78. In addition, AR-12 suppresses the ability of SARS CoV2 to produce virus spike protein and to generate infectious virion. AR-12 may hence be an antiviral against SARS CoV2 infection. [59]. Another study found imatinib as the top docking score drug in virtual screening on GRP78 nucleotide binding domain (NBD) [58].

GRP78 mediated damage of Beta cell of Pancreas

It had been reported that cytokine-exposed beta pancreatic cells cause secretion and cell surface translocation of GRP78 [60]. The csGRP78 can mediate cell signalling in pro-proliferative, pro-survival and pro-apoptotic pathways [47]. The secreted GRP78 itself acts as a ligand for csGRP78 on pancreatic β cell and activates the pre-apoptotic pathways leading to beta cell damage and hyperglycaemia [60]. COVID19 is associated with release of various cytokines depending on the stage and severity of the disease [40,41] and can cause hyperglycaemia via this pathway.

Summary

Increased incidence of CAMM during second wave of SARS CoV2 pandemic in India shows to have multifactorial causation. Hyperglycaemia, acidosis, impaired immunity and raised iron are important factors associated with pathogenesis of mucormycosis. Steroid therapy, raised ferritin and various factors unique to COVID19 viz. oxygen therapy, protective face masks, broad spectrum antibiotics and breached integrity of nasal mucosa have been implicated in creating a suitable environment for mucormycosis infection. Most CAMM cases had hyperglycaemia and history of glucocorticoid therapy.

The delta variant of SARS CoV2 and indiscriminate use of steroids were two unique features typical to second wave and appear to be drivers of CAMM. The endoplasmic reticulum chaperone GRP78 is over-expressed in viral infections and high viral load on RTPCR was observed in COVID19 during second wave. The higher viral load observed could be consequential to inadvertent glucocorticoids use, subsequently leading to higher ER stress and exaggerated GRP78 expression. Hyperglycaemia and raised ferritin also cause over-expression of GRP78.

Mucorales bind to host GRP78 via its CoTH proteins to invade tissues. On the contrary, these are not utilized by more common invasive fungi. Overexpressed csGRP78 is also a potential mediator of pancreatic beta cell damage leading to hyperglycaemia. Thus, hyperglycaemia is both cause and effect of over-expressed GRP78.

In COVID19 cases, phagocytic dysfunction can be caused by hyperglycaemia, glucocorticoids, raised ferritin and by virus itself. Owing to ubiquitous nature of Mucorales and the other implicated sources, peculiar to COVID19, escape from phagocytic destruction, upregulation of CoTH3 and availability of iron makes germination and tissue invasion possible in presence of abundant GRP78 receptors causing CAMM.

The limitation of this review in depicting comprehensive sequence of cellular events underlying CAMM necessitates further studies as published researches related to molecular mechanisms involved in its pathogenesis are scarce.

Conclusion

Phagocytic dysfunction, GRP78 over-expression, hyperglycaemia and ferritin derived iron are herein inferred as major determinants of mucormycosis in COVID19. Hyperglycaemia, both a cause and effect of GRP78 over-expression, has potentials of causing phagocytic dysfunction and CoTH3 upregulation in presence of acidosis. The delta variant and the rampant use of glucocorticoids qualify as most important factors for increased incidence of CAMM

during the second wave owing to their potential in generating the prerequisites.

In view of recurring waves and unpredictable nature of the SARS CoV2, there is compelling need and urgency for exploring anti-GRP78 agents for treatment of COVID19, mucormycosis and CAMM. The synergistic effects of anti-GRP78, anti-CoTH3 and anti-CoTH2 agents also need investigations for treatment of mucormycosis, an extremely dreadful fungal infection. Intensive research on molecular mechanisms involved in the pathogenesis of CAMM shall bridge the gaps in our current understanding for exploring targeted prevention and treatment options.

Conflict of interest

The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Spectrum of Behavioral Disorders Among Children Aged 5-15 Years in Tertiary Care Hospital of Bhopal

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Abstract

The current study was done to determine various behavioral disorders among 5-15 years of children attending Paediatric OPD and to determine underlying risk factors associated with behavioral disorders among children. This observational cross-sectional study was carried out among 200 children between 5-15 years of age using DSM IV Diagnostic Criteria. Some risk factors that lead to the manifestation of the disorder were also studied by history taking. The increasing sequence of disorders positivity was 0.5%, 1%, 1.5%, 1.5%, 2.5%, 12.5% for CD, LD, GAD, ADHD, AD and ODD, while observing 51.28% ODD cases among males, 12.5% ODD among overall subjects, 19.5% ADHD cases, 27.02% GAD, 1% LD and 0.5% CD cases. The overall observance of any one disorder was 39 (19.5%). Herein observed high prevalence of behavioral disorders requires behavioral, technical and managerial interventions and therefore is the priority intervention area in the field of public health globally, nationally and locally.

Key Words

Adolescent, Behavioral Disorders, Children, Mental Disorders, Public Health

Introduction

A mental disorder is a clinically significant behavioral or psychological syndrome or pattern that occurs in an individual and that is associated with present distress or disability or with a significantly increased risk of suffering, death, pain, disability, or an important loss of freedom. It has been underlined at Global level for appropriate intervention measures. (1-3) Mental disorders in childhood include Attention Deficit Hyperkinetic Disorder (ADHD), Oppositional Defiant Disorder (ODD), Autism Disorder (ASD), Generalized Anxiety Disorder (GAD), Learning Disorder (LD), Conduct Disorder (CD). It is often difficult to identify these problems due to lack of awareness. Child is often labeled as trouble maker, punished, ignored and ostracized by colleague's teachers and family members. Even parents find it difficult to deal with such issue. The future of the child is jeopardized. There is an urgent need to take few steps for better understanding of the problem and attend to it comprehensively (4). In India, the attempts to research in the field of mental health of school children are minimal. There are hardly any studies carried out to find the behavioral problems in children at the school level which is the crucial era of child's life. Therefore, the present study is conducted to assess the mental health of school going children. The study is cross sectional study involving the use of history of the patient and approved (American Psychiatric Association) diagnostic questionnaires. The proposed study focused to identify such neglected children and provide them their ability to channelize the energies towards constructive purposes so as to bring out the hidden potential in them. In India, the attempts to research in the field of mental health of school children are minimal (5-8). The study aimed to determine various behavioral disorders among 5-15 years of children attending Paediatric OPD and to determine underlying risk factors associated with behavioral disorders among children.

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Material and Methods

This observational cross-sectional study was carried out at pediatric OPD at People's College of Medical Sciences and Research Centre, Bhopal, MP. It was conducted during July-September 2018 after getting approval from IEC and ICMR. Study population comprising of 200 children included those between 5-15 years of age attending pediatric OPD. Approximately 50 patients report every day to the pediatric OPD selected for inclusion in the study. Those children aged 5-15 years were included in the study whose parents or guardians provided prior consent for inclusion. Exclusion criteria consisted of those not willing to participate in the study and critically ill patients. Information regarding Behavioral problems and Risk factors was gathered using DSM-IV diagnostic criteria (Diagnostic and Statistical Manual of Mental disorders. Data analysis was done by SPSS20 software.

Result

The study conducted for assessment of ADHD, ODD, ASD, GAD, LD and CD reveals remarkably high observance of ODD cases among Males [20 (51.28%) as compared to Females i.e., 05 (12.82%). However, the disorder observance in toto amongst the Males themselves included in the study was 84.61%. The overall percentage positivity rate is noted as 19.5% (*Table 1*). ODD is the only disorder in the study, which was found to have been represented across all predetermined age groups and the groups represented by symptoms. In addition, 60% of the cases identified for ODD have been found in the age group 5-8 years, whereas it is 36% and 4% for age group 9-12 years and 13-15 years respectively (*Table 2*). The study positivity for ODD was considered when there were 4, 5, 6, 7 or 8 symptoms present for at least 6 months in any of the age group considered for this study i.e., 5-8 years, 9-12 years or 13-15 years (*Table 3*).

The ASD as per DSM-IV diagnostic criteria was found in the age group 5-8 years, whereas no case was found among study population in the age group 9-12 years and 13-15 years (*Table 4*). There was no case in the age group of 13-15 years with overall 3 cases (1.5%) of the study population. (*Table 5*). The overall presence of learning disorder found in the study population was 1% (*Table 6*). The only case found among the whole population under study was having 7 symptoms as compared to minimum 3 symptoms required for identification of this disorder (*Table 7*). The overall observance of any one disorder among the listed and studied 6 disorders for the study population was 39(19.5%).

Table 1: Age and Gender wise Distribution of the Children Attendees of Tertiary Care Hospital for six Disorders

Age (Years)	Gender		ADHD		ODD		ASD		GAD		LD		CD		Total (a+b)	Total (c to n)
	M	F	M	F	M	F	M	F	M	F	M	F	M	F		
Column ID	a	b	c	d	e	f	g	h	i	j	k	l	m	n		
5 - 8	82	36	2	0	11	5	4	1	1	0	1	0	0	0	118	25
9 -12	56	20	1	0	8	0	0	0	2	0	1	0	1	0	76	13
13 -15	4	2	0	0	1	0	0	0	0	0	0	0	0	0	6	1
Total	142	58	3	0	20	5	4	1	3	0	2	0	1	0	200	39

ADHD: Attention Deficit Hyperkinetic Disorders; ODD: Oppositional Defiant Disorder; ASD: Autism Disorder; GAD: Generalized Anxiety Disorder; LD: Learning Disorder; CD: Conduct Disorder

Table 2: Observed 'Attention Deficit Hyperkinetic Disorders' (ADHD) as per DSM-IV Diagnostic Criteria among Children Attendees of Tertiary Care Hospital

Age Group (Years)	Number of Symptoms for at least Six Months					Total	
	≤ 5	6	7	8	9		
	a	b	C	d	e	b to e	a to e
05 – 08	116	1	1	0	0	2	118
09 – 12	75	0	1	0	0	1	76
13 – 15	06	0	0	0	0	0	06
Total	197	1	2	0	0	3	200

Table 3: Observed 'Oppositional Defiant Disorder' (ODD) as per DSM-IV Diagnostic Criteria among Children Attendees of Tertiary Care Hospital

Age Group (Years)	Number of Symptoms for at least Six Months						Total	
	≤ 3	4	5	6	7	8		
	a	b	c	D	e	f	b to f	a to f
05 – 08	103	4	5	3	3	0	15	118
09 – 12	67	5	2	0	1	1	9	76
13 – 15	05	1	0	0	0	0	1	06
Total	175	10	7	3	4	1	25	200

Table 4: Observed 'Autism Disorder' (ASD) as per DSM-IV Diagnostic Criteria among Children Attendees of Tertiary Care Hospital

Age Group (Years)	Number of Symptoms Present								Total	
	≤ 5	6	7	8	9	10	11	12		
	a	b	c	d	E	f	g	h	b to h	a to h
05 - 08	113	4	1	0	0	0	0	0	5	118
09 - 12	76	0	0	0	0	0	0	0	0	76
13 - 15	06	0	0	0	0	0	0	0	0	06
Total	195	4	1	0	0	0	0	0	5	200

Table 5: Observed 'Generalized Anxiety Disorder' as per DSM-IV Diagnostic Criteria among Children Attendees of Tertiary Care Hospital

Age Group (Years)	Generalized Anxiety Disorder	
	Present	Absent
05 – 08	1	117
09 – 12	2	74
13 – 15	0	06
Total	3	197

Table 6: Observed 'Learning Disorder' as per DSM-IV Diagnostic Criteria among Children Attendees of Tertiary Care Hospital

Age Group (Years)	Learning Disorder	
	Present	Absent
05 – 08	1	117
09 – 12	1	75
13 – 15	0	06
Total	2	198

Table 7: Observed 'Conduct Disorder' as per DSM-IV Diagnostic Criteria among Children Attendees of Tertiary Care Hospital

Age Group (Years)	Number of Symptoms for at least Six Months														Total	
	≤ 2	3	4	5	6	7	8	9	10	11	12	13	14	15		
	a	b	c	d	e	f	g	H	i	j	k	l	m	n	b to n	a to n
05 – 08	118	0	0	0	0	0	0	0	0	0	0	0	0	0	0	118
09 – 12	75	0	0	0	0	1	0	0	0	0	0	0	0	0	1	76
13 – 15	06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	06
Total	199	0	0	0	0	1	0	0	0	0	0	0	0	0	1	200

Discussion

In view of the unaddressed underlined needs of comprehensive interventional frameworks for behavioral disorders and to appropriately address co-morbid conditions as well, the current study results agree with the inferences drawn by various studies (9-11). Unlike the overall percentage positivity of behavioral disorders in the children aged 5-15 years observed as 19.5% in the current study, the prevalence of behavioral disorders is

assessed to be 5.13% in North Carolina (4), 12.5% in Bengaluru, 6.3% in Chandigarh and 5-6% in other States of India (6). Noticeably high prevalence (42%) of behavioral problems with no gender difference was observed in a study from Delhi (7). Hence, this study also identifies focused areas for dealing with behavioral disorders especially ADHD and LD through capacity building of families of the affected children and adolescent like other studies viz. Indian study (5) and Taiwan based study (12). The inference of current study for overall positivity being 19.5% for behavioral disorders is in resonance with those observed in Canada, Germany and USA being 18.1%, 20.7% and 21% respectively (13, 14). The present study agrees with the observation of U.K., Southern Illinois University based studies and other important studies (2,15,16,17) for the need to address socio-economic indicators of health and developing a strong network of public health approach-based diagnostic, treatment and care cum support solutions. The prevalence of Psychiatric disorders in young adolescents of Central China (18) was assessed to be 9.74% with ADHD, ODD and GAD being 4.96%, 2.98% and 1.77% respectively. They also found ADHD to have co-morbidity of 25.15 % with ODD, 18.18% with CDD and 6.38% with GAD. The questionnaire used in the current study was as per DSM-IV criteria, which has been used extensively and in standardized clinical settings for assessment of behavioral disorders (19). However, the studies based on some other scale viz. Rutter score (8), CBCL 1.5-5 ADH Problems Scale (14), Dutch Norms for the Strength and Difficulties Questionnaire (16) or SCARED scale (17) may also interfere with the comparability of observations across various studies. SCARED is closely linked to DSM classification and hence can be used for assessment of disorders and required guidance cum treatment. The prevalence of behavioral disorders in the current study had high preponderance of male cases (97.43%) as compared to other studies showing it to be double in the males as compared to females (20).

The conduct of present study was undertaken at the tertiary health care center of Central India and it being the health care service provider of excellence may also include a large number of referred cases from the primary and secondary health care facilities. This may be one of the reasons for prevalence herein being more than those mentioned in the referenced studies in addition to other factors such as difference in the design, sample size and nature of conducted studies.

Conclusion

The high prevalence of behavioral disorders including ADHD, ODD, ASD, GAD, LD and CD is assessed here and therefore requires behavioral, technical and managerial priority intervention area in Public Health. Male preponderance of behavioral disorders also requires careful and timely attention of policy makers, programme planners and activity implementers through government, semi-government and private service, care and support provisions.

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