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Case Report

Bilateral Neck of Femur Pathological Fracture in Young Female with Coxa Vara and Psoriasis Vulgaris - A Case Report

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Abstract

Introduction: Most of the hip fractures occur in elderly population, with 50% consisting of femoral neck fractures. In these patients, hemiarthroplasty or total hip replacement comprises the main line of management for early mobilization. However, 3–10% of these fractures occur in young adults where the main goal of treatment is to preserve the natural hip anatomy and biomechanics due to high functional demands.

Case Report: Here, we present a rare case of bilateral pathological neck of femur (NOF) fracture in young adult female, a known case of psoriasis vulgaris and developmental coxa vara. Patient was operated with cancellous cannulated screws and dynamic hip screw plating with good functional hip range of motion and immediate full weight bearing walk with walker.

Conclusion: In psoriatic patients, psoriasis vulgaris coexists with confluence of other psoriatic disorders and comorbidities, which result in osteoporosis, hypovitaminosis D, decreased bone mineral density, and propensity for fragility fractures mainly involving NOF.

Key words: Bilateral, Insufficiency fractures, Neck of femur, Osteomalacia, Pathological fractures, Psoriasis vulgaris, Stress fractures, Young female

INTRODUCTION

Most of the hip fractures occur in elderly population, with 50% consisting of femoral neck fractures. In these patients, hemiarthroplasty or total hip replacement comprises the main line of management for early mobilization. However, 3–10% of these fractures occur in young adults where the main goal of treatment is to preserve the natural hip anatomy and biomechanics due to high functional demands.[1]

Normal levels for serum Vitamin-D are >20 ng/ml, calcium >9.0 mg/dl, alkaline phosphatase 115–359 U/L, and parathyroid hormone 160–520 pg/ml.[2]

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Risk factors such as excessive alcohol abuse and tobacco consumption with deficiency in serum Vitamin-D levels causing severe osteomalacia due to inadequate sunlight (ultraviolet B) exposure, decreased intake in diet, malabsorption of Vitamin-D, or kidney and liver disorders play important role in such pathological neck of femur (NOF) fractures.[2,3]

Osteoporosis at NOF has a prevalence of 5% and low bone mass having 39%. Patients with sudden onset hip pain without any trauma and absent X-ray findings should be evaluated for serum Vitamin-D, alkaline phosphatase, and bone mineral density by bone scan or MRI to screen for pathological NOF fractures.[2,4]

There are various modalities of treating NOF fractures such as conservative management with derotation boot in elderly unfit patients and surgical treatment such as cannulated screws, dynamic hip screw, proximal femur nail, angled blade plates, dynamic condylar screw, and hip replacement surgeries.[5]

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Psoriasis is commonly seen in 50–69 years age group with prevalence being 1–3%. Recent literature supports the finding of increased risk of osteoporosis, decreased bone mineral density, and fragility fractures in psoriatic disease but the evidence for strength of association remains inconclusive. However, there has been definite association of psoriasis and fragility fractures. Hence, preventive measures for fragility fractures must be considered in these patients.[6]

CASE REPORT

A 24-year-old female came to our OPD with sudden onset left hip pain since 1 month without any history of trauma. Patient is unable to walk since. Patient has history of operated for right NOF fracture by cannulated screws 4 years back after a similar trivial trauma. She was a known case of psoriasis vulgaris, on medications and on X-rays; patient had an old left NOF fracture is shown in Figure 1. On blood reports, patient had serum Vitamin-D3 deficiency with value of 8 ng/ml.

Patient was posted for surgery after pre-anesthetic fitness.

Surgical Procedure

Patient was taken on operating traction table under all aseptic precautions and under epidural plus spinal anesthesia scrubbing, painting, and draping done. Reduction checked under C-arm after traction and internal rotation of hip. Lateral approach to hip with incision started at tip of greater trochanter extending distally along shaft of femur laterally about 10 cm. Soft-tissue dissected and tensor fascia lata incised along the incision. Vastus lateralis elevated from vastus ridge anteriorly after inverted L shaped incision. First guide wire was inserted inferiorly through calcar. Other two guide wires inserted anterosuperior and posterosuperior to it in an inverted triangle configuration. Triple reamer used to drill through first guide wire. Richard's screw of size 80mm is then inserted. Two cancellous cannulated screws 60 mm each were then crossed through fracture site. Traction released. Final tightening of Richard screw with top nut done. Both cannulated screws then tightened. Wound wash given with betadine solution and normal saline. Deep closure is done with vicryl 1 and 2.0. Skin closed with ethilon 2.0.

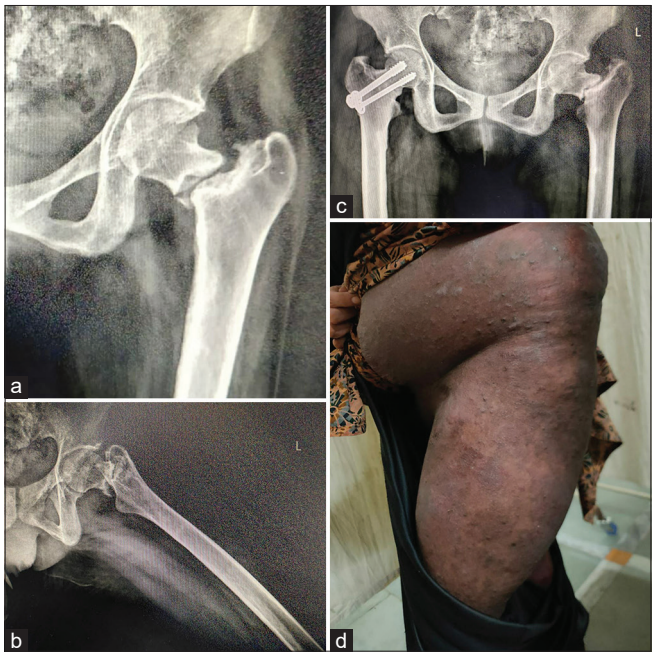

Figure 1: (a-d) Pre-operative X-rays showing the left neck of femur fracture in anteroposterior and lateral views with operative site showing psoriatic vulgaris skin lesions. Previously, operated the right neck of femur fracture with cannulated cancellous screws

Patient was full weight bearing with walker from post-operative day 2 with good functional range of hip motion.

Patient was started on weekly Vitamin-D3 60K capsules for 12 weeks.

Check dress done on day 3 and 7 found to be healthy with suture removal done at day 12. Follow-up X-rays at post-operative 8 weeks show good fracture union, satisfactory hip range of motion and full weight bearing walk without walking aide with ease of ground level activities such as squatting and cross legged sitting, as shown in Figures 2 and 3.

DISCUSSION

About 10–30% of non-union rates and 15–30% of osteonecrosis rates are observed after NOF fractures. Age and fracture displacement play an important role in determining these complications. Early literature suggested surgical reduction and fixation within 6–24 h after trauma determined better outcome whereas recent studies found no such correlation with time of surgery after trauma and femur head ischemia or non-union.[7]

Physiological age of the patient taking into account the pre-trauma activity level, comorbidities, bone strength, and quality determines the choice of management.[1]

Femoral head blood supply comes from medial circumflex femoral artery (MCFA), lateral circumflex femoral artery, and obturator artery. Of these, the majority of femur head and main weight bearing superolateral aspect are supplied by lateral epiphyseal branch of MCFA. In displaced NOF fractures, this artery gets damaged at postero-superior

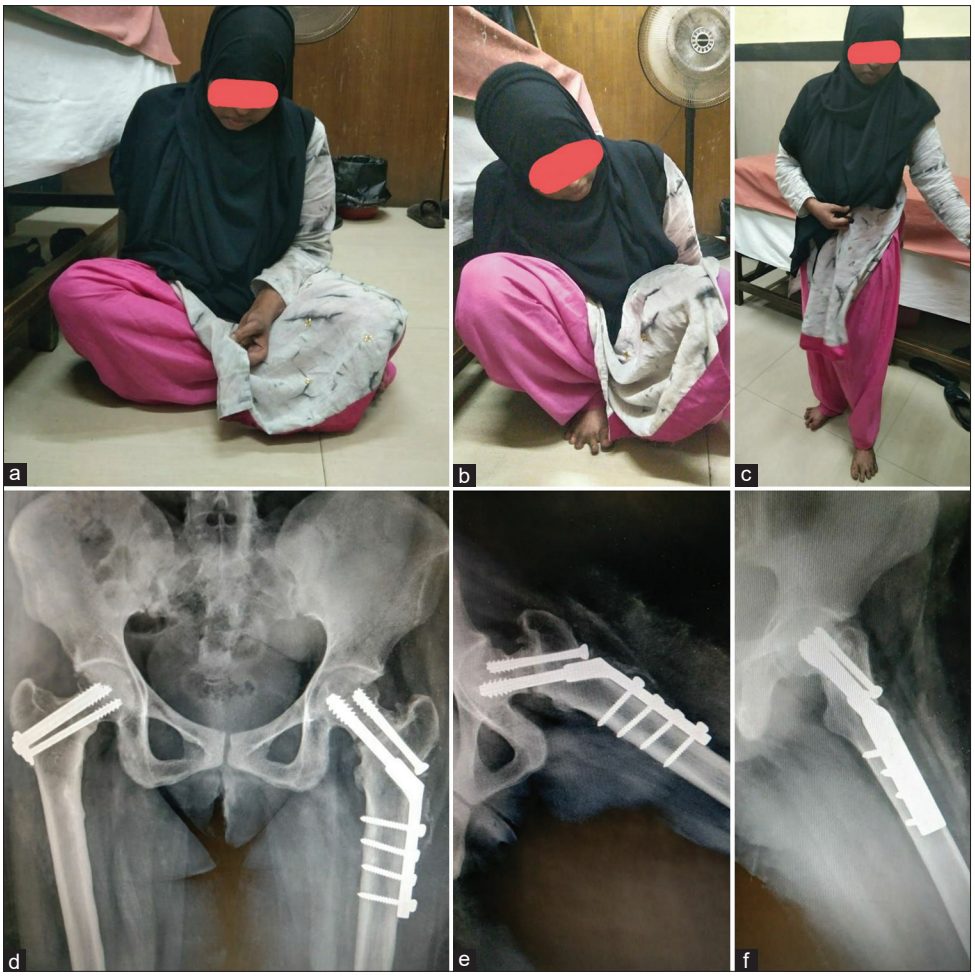

Figure 2: (a-f) Post-operative X-rays at 2 months showing good fracture union and full weight bearing walk without walking aide with ease of ground level activities such as squatting and cross legged sitting

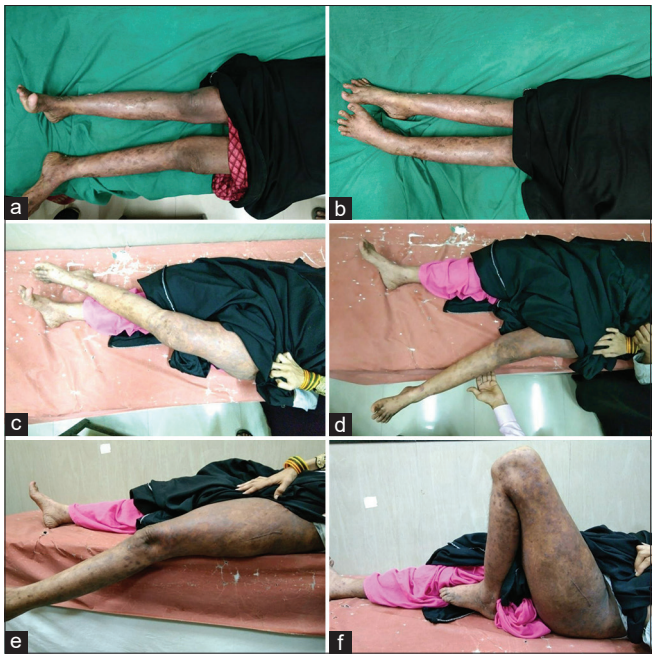

Figure 3: (a-f) Clinical pictures at 2 months post-operative showing satisfactory hip range of motion

aspect of NOF. In NOF fractures, the disruption of intracapsular arteries along with increased intracapsular pressure due to fracture hematoma is hypothesized as the reason for femoral head ischemia. Hence, pre-operative traction and surgical reduction lead to restoration of blood flow to head.[1]

Stress fractures of proximal femur are seen commonly in athletes and military trainees. They are further classified as insufficiency fractures occurring due to normal stress on weakened bone with associated pathological disorders and fatigue fractures occurring in normal bone due to excessive physical activity. Insufficiency fractures are commonly seen in patients with osteomalacia where there is decreased bone mineralization due to deficient serum Vitamin-D.[5]

A hip deformity with decreased neck shaft angle of <120° is termed as coxa vara which leads to deranged hip biomechanics due to concentration of focal mechanical stress in NOF which makes it vulnerable to stress fractures.[5]

Insufficiency or pathological NOF fractures have been commonly seen in conditions such as osteomalacia, pregnancy, pelvic irradiation, corticosteroid abuse, chronic renal disease, metabolic bone disease, and psoriasis vulgaris.[2]

These fractures in young adult compromise the femoral head blood supply lead to high probability of osteonecrosis and non-union even after internal fixation especially in displaced NOF fractures. This mandates anatomic reduction and stable internal fixation.[1]

In young adults, NOF fractures occur usually as a result of high energy trauma but in few cases of pathological disorders, trivial fall can also lead to NOF fractures.[7,8]

In rheumatic diseases such as psoriatic arthritis, bone strength is decreased by both the disease processes producing pro-inflammatory cytokines which promote osteoclastogenesis and destruction of arthritic bone. The immunosuppressive corticosteroids further weaken the bone strength. In psoriatic arthritis, there are synovial hypertrophy and hyperplasia with increased angiogenesis, inflammatory cell infiltrates and are negative for anti-citrullinated proteins.[9]

In the literature, osteoporosis has been documented in psoriatic arthritis patients with significantly low T and Z scores by Riesco and Manzano *et al.*[9]

Kocijan *et al.* stated that trabecular density was significantly decreased in psoriasis patients. There also has been increased prevalence of hypovitaminosis D, high bone turnover, and low bone mineral density pre-disposing to fragility fractures in psoriatic patients.[10]

CONCLUSION

In psoriatic patients, psoriasis vulgaris coexists with confluence of other psoriatic disorders and comorbidities, which result in osteoporosis, hypovitaminosis D, decreased bone mineral density, and propensity for fragility fractures mainly involving NOF. In these patients, the decreased bone quality further leads to focal stress and developmental coxa vara which further raises the fracture risk. Hence, in psoriatic patients, preventive measures must be undertaken for pathological fractures and bilateral hip radiological screening must be undertaken for any spontaneous hip pain with Vitamin-D3 supplementation and adequate sunlight exposure.

REFERENCES

1. Gupta A, Choudhari P, Nagla A. Management of femoral neck fractures in young/middle aged adults. Orthop J MP Chapter 2018;24:48-60.
2. Fukui K, Kaneuji A, Hirata H, Tsujioka JI, Shioya A, Yamada S, *et al*. Bilateral spontaneous simultaneous femoral neck occult fracture in a middle-aged man due to osteoporosis and vitamin D deficiency osteomalacia: A case report and literature review. Int J Surg Case Rep 2019;60:358-62.
3. Yoon BH, Kwon MS. Atraumatic bilateral fracture of the femoral neck in young male patient with suspected osteomalacia. J Bone Metab 2017;24:197-200.
4. Borrud LG, Dawson-Hughes B, Looker AC, Shepherd JA, Wright NC. Osteoporosis or low bone mass at the femur neck or lumbar spine in older adults, United States, 2005-2008. NCHS Data Brief 2012;93:1-8.
5. Sariyilmaz K, Ozkunt O, Sungur M, Dikici F, Yazicioglu O. Osteomalacia and coxa vara. An unusual co-existence for femoral neck stress fracture. Int J Surg Case Rep 2015;16:137-40.
6. Chen TL, Lu JW, Huang YW, Wang JH, Su KY. Bone mineral density, osteoporosis, and fracture risk in adult patients with psoriasis or psoriatic arthritis: A systematic review and meta-analysis of observational studies. J Clin Med 2020;9:3712.
7. Gümüştaş SA, Tosun HB, İşyar M, Serbest S, Öznam K, Bulut G. Femur neck fracture in young adults, is it really an urgent surgery indication: Retrospective clinical study. Pan Afr Med J 2018;30:112.
8. Sadat-Ali M, Ahlberg A. Fractured neck of the femur in young adults. Injury 1992;23:311-3.
9. Riesco M, Manzano F, Font P, García A, Nolla JM. Osteoporosis in psoriatic arthritis: An assessment of densitometry and fragility fractures. Clin Rheumatol 2013;32:1799-804.
10. Pethő Z, Kalina E, Pap Z, Hodosi K, Falcsik R, Balogh Á, *et al*. Characterization of bone metabolism in Hungarian psoriatic arthritis patients: A case control study. BMC Musculoskeletal Disord 2021;22:1-9.

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Case Report

Microimplant-assisted Orthodontic Correction of Class I Bimaxillary Protrusion with Gummy Smile: A Case Report

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Abstract

Orthodontic management of Class I severe bidental protrusion with incompetent lip and gummy smile has always been a clinical challenge. Simple orthodontic biomechanics can bring compromised esthetic outcome and lacks three-dimensional control of the dentofacial deformity. Although a combination of orthodontics with orthognathic surgery may be the ideal approach, the complications, risks, and costs of surgery have stimulated interest in alternative treatment methods. In recent years, bone borne temporary anchorage devices such as endo-osseous implants, miniplates, mini-implants, microimplants, and screws have opened a new era in clinical orthodontics by revolutionizing the way anchorage is controlled. Microimplants can now be used as effective anchors by simultaneously intrusion and retraction of the upper anterior teeth to mimic a maxillary anterior subapical osteotomy. The aim of the case report is to show the efficiency of microimplant-assisted orthodontic correction of a severe and complicated malocclusion.

Key words: "Gull wing lip" deformity, Intrusion, Microimplant, Retraction

INTRODUCTION

Class I bimaxillary protrusion with incompetent lip is a common dentofacial deformity. If the differences between philtrum length and commissural length of the upper lip are more than 10 mm, it is called "Gull Wing Lip" deformity,[1] as it resembles the wing of flying Sea gull. In the present case, conventional treatment plan is orthognathic surgery like maxillary anterior subapical osteotomy.

Microimplants are intraoral skeletal anchorage systems which provide absolute or perfect anchorage control, thus widening the envelope of orthodontic treatment.[2] These have now become an indispensable tool in clinical orthodontics. Both the prosthodontic and the orthodontic implants are composed of titanium but the orthodontic implants are to be used temporarily whereas the prosthodontic implants are to be used permanently. There is axial loading for the prosthodontic implants but the type of loading for the orthodontic implants is non-axial. The diameter of the implants used for prosthodontic purposes is larger than that used for the orthodontic purposes.[3]

Microimplants are most often used because of their advantages such as tiny size, minimally invasive, easy placement and removal, ability to withstand immediate loading, placement at various anatomic locations, and low cost and do not require patient cooperation.[4] Orthodontic mini-implants or microscrew implants are non-osseointegrated monocortical or bicortical titanium alloy screws, ranging from 6 to 12 mm in length, and 1.2–-2 mm in diameter,[5] fixed to bone temporarily to enhance orthodontic anchorage.

For orthodontic retraction, Hickham (1978) once stated "You have to get them up to get them back." Hence, microimplant-assisted true intrusion and retraction were selected for orthodontic correction of Class I bidental protrusion with "Gull Wing lip" deformity.

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CASE REPORT

A 13-year-old female patient of Bengalee origin reported to the department of orthodontics with the chief complaint of protruding front teeth. Clinical examination and preliminary investigation revealed mesoprosopic facial form with convex profile and incompetent lip. Eight millimeters incisor display at rest and full incisor display with 3 mm gingival display on smile were found. The differences between philtrum length and commissural length were 14 mm, signify well-established "Gull Wing Lip" deformity [Figure 1].

It was a case of Class I bimaxillary protrusion with increased overjet, deep, and complete overbite. Lower arch was showing significant anterior crowding. The patient had significant anterior dentoalveolar proclination. Skeletally, the patient had Class II skeletal base and average growth pattern.

Orthodontic treatment started with extraction of all first premolars. Straight wire mechanotherapy started with 0.022 MBT system. After completion of leveling and aligning of upper arch, four microimplants were placed bilaterally, two between 2nd premolar and 1st molar, two between lateral incisor and canine [Figure 2].

Microimplants used were Aarhus type microimplant made of titanium alloy (S.K. Surgical), 1.5 mm in diameter and 6 mm in length. After following the disinfection protocol, microimplants were placed under local infiltration anesthesia. An infiltration of 0.5 ml of local anesthesia (Xylocaine 2%, 1:80,000 Lignox, Indoco Remedies Ltd.) was administered in each four region of labial and buccal vestibule of maxilla. Predrilling with pilot drill of 1 mm diameter is done before placement of the microimplant. Using hand guided microimplant handpiece, microimplants were inserted in the interdental bone in rotating motion, maintaining minimum insertion torque. Between the 2nd premolar and 1st molar microimplants were placed 5 mm above the interdental papilla, between lateral incisor and canine, it was placed 8 mm above the interdental papilla. Microimplants were placed in the attached gingival. Microimplants were placed in an oblique direction

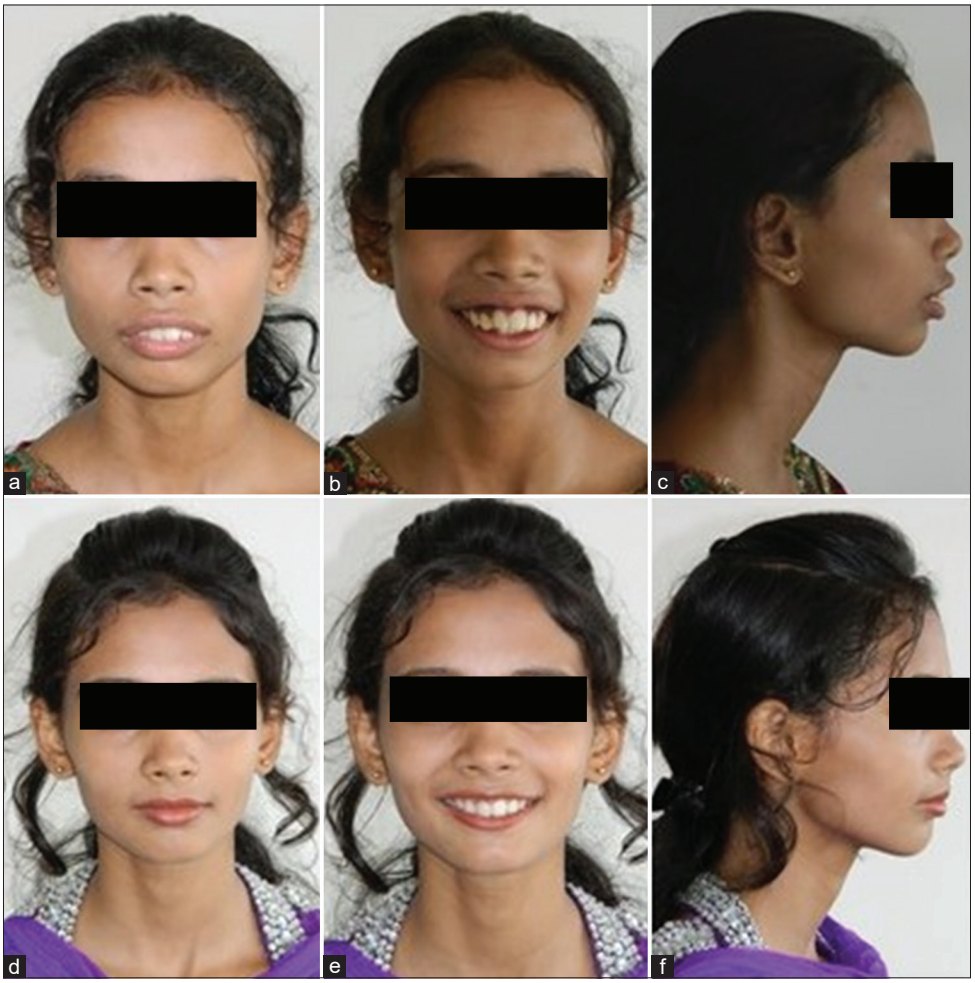

Figure 1: (a-f) Comparison of pre- and post-treatment extraoral photograph

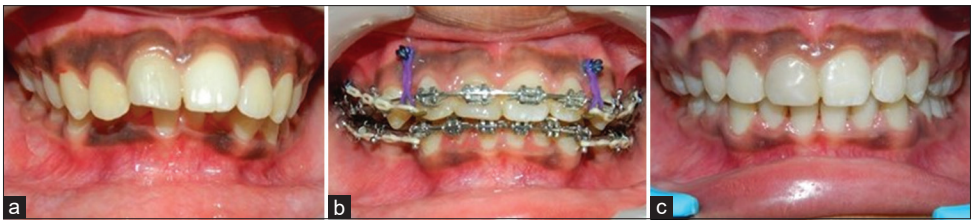

Figure 2: (a-c) Comparison of pre-treatment, mid-treatment, and post-treatment intraoral photograph

buccolingually 40°[6] to the long axis of the teeth and microimplants were inclined distally about 20°and placed 1 mm distally to the contact point[7] to prevent injury to root and to cover maximum cortical bone for better stability.[8]

Immediate loading of intrusion and retraction forces was done from small hooks attached to the archwire to microimplants. Intrusion force was 50 mg each side and retraction force was 150 mg each side. Archwire size was 19 × 25 ss. Archwire was customized with buccal crown torque to incisors to prevent lingual tipping. Archwire was given mild constriction in premolar region to prevent widening effect of retraction using microimplants.

Lower arch leveling was done by segmental mechanism and Burstone intrusion arch. Regular checkup was done in every 6 weeks. Completion of treatment occurred in 2 years. After finishing and detailing procedure, microimplants were removed under topical anesthesia. After significant incisor, intrusion has been achieved, gingival recontouring might be needed for increasing the clinical crown height particularly in gummy smile patients. However, in the present case, only periodic periodontal evaluation and oral prophylaxis were done.

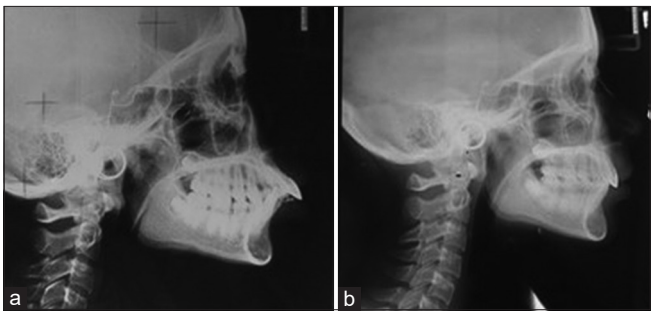

Figure 3: (a and b) Comparison of pre- and post-treatment cephalogram

Parameters	Pretreatment	Posttreatment
Upp. incisor APog	16mm	5mm
Upp. incisor to NA	31degree/8.5mm	19degree/4.5mm
Upp. INCISOR to point A	16mm	5mm
Lower incisor to NB	45degree/11mm	28.5degree/4.5mm
Upp incisor to NF	8mm	4mm
Occlusal plane to SN	19 degree	19 degree
FMA	25 degree	23degree
Nasolabial angle	104.5degree	115degree
E-Line	U Lip +5mm/L Lip +9mm	U Lip +1mm/L Lip +2mm

Chart 1: Comparison of pre-treatment and post-treatment cephalometric data

RESULTS

After completion of treatment, balanced facial profile with pleasing esthetics is achieved. Lip competency at rest is restored. On smile, full incisor display without any gum display is seen. Reduction in lip strain is achieved [Figures 1 and 2]. Cephalometric analysis showing significant improvement in E-line, S-line, and nasolabial angle [Figure 3]. Upper incisor to NA reduced to 4.5 mm/20° from initial value of 8.5 mm/31°. Four millimeters upper incisor intrusion in relation to nasal floor is measured. Two degree decrease in mandibular plane angle is seen, which may be due to slight intrusion of posterior segment of dentition (chart 1).

DISCUSSION

Intrusion of upper anterior teeth is helpful in correcting upper incisor display and to reduce "gummy smile." It occurs due to vertical maxillary excess, deep bite due to supraeruption of upper anterior teeth and short upper lip ("Gull Wing lip"). Since the lip morphology is directly related to the morphology of the vestibular sulci,[9] the answer to the correction of gull wing lip morphology is to produce torque controlled true intrusion and retraction of upper incisors. Intrusion brings the incisors into a position where more spongeous bone for tooth movement is available – this allows for greater range of retraction.

For every 1 mm of retraction, there is 0.5 mm reduction in the interlabial gap, when retraction is not associated with either intrusion or extrusion of incisors. When retraction is associated with intrusion of incisors, every 1 mm of retraction reduces the interlabial gap proportionately by 1 mm. When retraction is associated with extrusion of incisors, 1 mm of retraction does not reduce the interlabial gap.[10]

Vertical lip height increases while dental height decreases with treatment. The increase in vertical lip height is significantly dependent on the reduction in overjet.[11] During orthodontic intrusion of incisors in patients with intact periodontium, crown height reduction is less and gingiva also follows the intrusion. The gingival margin moves apically 79% and the mucogingival junction moves apically 62% of total intrusion,[12] provided that there was adequate control of bacterial plaque. These results were also found to be considerably stable in long term.

Since it is biomechanically difficult to produce true intrusion and retraction with regular orthodontic mechanics, as all intrusion arches cause extrusion and distal tipping of molars. Flaring of incisor also occurs, which, in turn, restricts anterior intrusion due to close approximation of root to the palatal cortical bone. Rigid microimplant-supported anchorage with two in the anterior and two in the posteriors dentoalveolar segment is used to intrude and retract the incisors simultaneously, in the present case. Usually, a force originating from a single microimplant placed between the maxillary central incisor roots is adequate to intrude the anterior dentition. However, this may lead to a transverse cant of the occlusal plane. To minimize this, two microimplants are placed bilaterally between the roots of lateral incisor and canine. For retraction, two microimplants are placed bilaterally between upper 1st molar and 2nd premolar. It is the safest zone in the inter-radicular space of the posterior maxilla.[13]

The incisor retraction with mini-implants primarily was achieved by controlled tipping and partly translation because the forces applied were closer to center of resistance of maxillary teeth.[14] About 7 mm of bodily retraction was can be achieved.[15] Palatal cortical bone and width of alveolar bone could be limiting factors in incisor retraction.[16] Hence, forces used for retraction must be extremely physiologic (150–200 g/side).

Bodily tooth movement or root movement requires higher anchorage value than controlled/uncontrolled tipping. Retraction force from short hooks attached to archwire, the line of force runs below the center of resistance. It produces controlled tipping of anteriors when archwire fits passively to the anterior brackets. When the force is reduced to 100 g and additional labial crown torque is applied to the archwire in the anterior segment, a constant and light intrusive force from microimplant prevent extrusion or labial flaring of incisor. It causes "pure" root movement while maintaining the position of the incisor tip.[5]

In microimplant-assisted intrusion and retraction, relapse of anterior teeth retraction ranges from 10 to 15% and anterior intrusion is 25 to 30%,[17] so slight overcorrection is required. However, microimplants have some disadvantages. Apical root resorption of maxillary incisors is 16.5–19.8% treated with miniscrews intrusion.[18] It is significantly more than patients treated without miniscrews, which is 5.4%. This is due to longer retraction time required in patient treated with miniscrew. Lighter intrusion forces in the range of 60–120 g (10–20 g per tooth) are applied for proper intrusion of anterior teeth and to minimize root resorption.

CONCLUSION

- In severe bidental protrusion with gummy smile, better esthetic outcome can be achieved with microimplant-assisted orthodontic treatment
- In the present case, true intrusion and bodily retraction of upper anteriors are achieved
- An "Orthognathic-like Orthodontic" treatment outcome secures the patient with optimum smile esthetics.

REFERENCES

1. Epker BN, Stella JP, Fish LC. Dentofacial Deformities: Integrated Orthodontic and Surgical Correction. Vol. 1. United States: Mosby; 1995. p. 19.
2. Kanomi R. Mini-implant for orthodontic anchorage. J Clin Orthod 1997;31:763-7.
3. Wehrbein H, Merz BR, Hammerle CH, Lang NP. Bone-to-implant contact of orthodontic implants in humans subjected to horizontal loading. Clin Oral Implants Res 1998;9:348-53.
4. Lin JC, Yeh CL, Liou EJ, Bowman SJ. Treatment of skeletal origin gummy smiles with miniscrewanchorage. J Clin Orthod 2008;42:285-96.
5. Nanda R, Uribe FA. Temporary Anchorage Device. United States: Mosby, Elsevier; 2009.
6. Park HS, Bae SM, Kyung HM, Sung JH. Micro-implant anchorage for treatment of skeletal Class I bialveolar protrusion. J Clin Orthod 2001;35:417-22.
7. Park HS, Hwang ES, KwonTG. Proper mesiodistal angles for microimplant placement assessed with 3-dimensional computed tomography image. Am J Orthod Dentofacial Orthop 2010;137:200-6.
8. Kuroda S, Yamada K, Deguchi T, Hashimoto T, Kyung HM, Yamamoto TT. Root proximity is a major factor for screw failure in orthodontic anchorage. Am J Orthod Dentofacial Orthop 2007;131:S68-73.
9. Arnett GW, Bergman RT. Facial keys to orthodontic diagnosis and treatment planning-Part II. Am J Orthod Dentofacial Orthop 1993;103:395-411.
10. Jacobs JD. Vertical lip changes from maxillary incisor retraction. Am J Orthod Dentofacial Orthop 1978;74:396-404.
11. Mamandras AH. Linear changes of the maxillary and mandibular lips. Am J Orthod Dentofacial Orthop 1988;94:405-10.
12. Erkan M, Pikdoken L, Usumez S. Gingival response to mandibular incisor intrusion. Am J Orthod Dentofacial Orthop 2007;132:143.e9-13.
13. Pajongiit C, Boonsiva S, Eduardo YS. "Safe Zones" for miniscrew implant placement in different dentoskeletal pattern. Angle Orthod 2011;81:397-403.
14. Upadhay M, Yadav S, Nagraj K, Patil S. Treatment effect of miniimplants for enmass retraction of anterior teeth in bialveolar dental protrusion patient: A randomized controlled trial. Am J Orthod Dentofacial Orthop 2007;134:18-29.e1.
15. Park HS, Kwon TG. Sliding mechanics with microscrew implant anchorage. Angle Orthod 2004;74:703-10.
16. Horiuchi A, Hotokezaka H, Kobayashi K. Correlation between cortical plate proximity and apical plate resorbtion. Am J Orthod Dentofacial Orthop 1998;114:311-8.

17. Hsu SP, Liou EJ. Stability Evaluation of en Masse Maxillary Retraction and Intrusion by Using Miniscrews: One Year Follow-Up, Taiwan Association of Orthodontists Annual Meeting; 2005.

18. Liou EJ, Chang PM. Apical root resorption in orthodontic patients with en-masse maxillary anterior retraction and intrusion with miniscrews. Am J Orthod Dentofacial Orthop 2010;137:207-12.

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Original Article

Augmentation of Volar Locking Plate with Percutaneous Kirschner Wires in Management of Unstable Distal Radius Fractures

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Abstract

Background: Distal radius fractures are common and are increasing in incidence, especially in older age group and younger population. There is spectrum of methods of treatment available ranging from closed reduction to various surgical procedures, with each one has its own benefits and complications. Our technique involves open reduction and internal fixation with locking plate (LCP) and augmentation of the fracture with percutaneous Kirschner wire (K-wire), and POP immobilization of the unstable distal radius fracture for 4–6 weeks. This study examines the functional outcome of volar plating with K-wire augmentation in unstable distal radius fractures.

Material and Methods: This is a prospective study conducted in a tertiary care hospital of 48 patients aged between 21 years and 71 years, with unstable distal radius fracture. Patients were treated by open reduction and internal fixation with LCP and percutaneous pinning using two to three K-wires to maintain radial inclination and stability of radial styloid fracture fragment. The wires are cut and bent to the outside. A dorsal below elbow POP slab was applied for 4–6 weeks. K-wires were removed at interval of 6–8 weeks. All the patients were followed up at regular intervals of 3 weeks, 6 weeks, 12 weeks, and 24 weeks. The functional evaluation was done at 24 weeks follow-up. We applied Sarmiento's modification of Lindstrom criteria and Gartland and Warley criteria for the evaluation of results.

Results: Excellent to good results were seen in 83.33% of cases, fair results in 15.73%.

Conclusion: Augmentation with percutaneous pinning of LCP is a simple, functionally effective, safe method to maintain the fracture reduction of the principle columns of distal radius, it is easily reproducible and has less interference of the soft tissue, compared to the standard dual plating method which is used routinely.

Key words: Distal radius fractures, K-wire augmentation, Locking plate

INTRODUCTION

Fracture of the distal radius and ulna is the most common fracture encountered by orthopedic trauma surgeons around the world. The history of fractures of the distal radius reflects the evolution of the understanding of many conditions in orthopedic trauma. Fractures of the distal radius were brought to the attention of the English speaking literature in 1814 when Abraham Colles published his views "On the fracture of the carpal extremity of the radius" in 1814.[1]

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At present, for the management of distal radius fracture, a spectrum of modalities is available ranging from closed reduction and immobilization to open reduction and internal fixation, as present knowledge stands, we cannot predict the outcome of a distal radius fracture with any degree of confidence but we can only recommend levels of displacement which can be accepted in the fit, active, and fully functioning patient. Selection of patient for a particular treatment is very important and should be given meticulous care, broadly, the patient selection should depend on metaphyseal stability, articular involvement, and displacement of the fracture at the time of presentation.[2,3]

According to the "three-column theory" of distal radius fractures proposed by Rikli and Regazzoni. Distal radius and ulna fractures are classified under the columnar classification system it further divides the distal radius and

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ulna into three columns: (1) Lateral distal radius (radial styloid and scaphoid facet), (2) medial distal radius (lunate facet), and (3) the ulnar column (TFCC and ulnar styloid).[1-3]

In this study, we aim to analyze radiological and clinical outcome in unstable distal end radius fracture managed with volar plating and Kirschner wire (K-wire) augmentation; what constitutes distal radius "unstable" fracture, there is no firm description of unstable radius fracture, however, we can say that instability, or the inability of a fracture to resist displacement after closed reduction, is often defined according to criteria regarding initial displacement, such as dorsal angulation, shortening, and the presence of dorsal comminution.[4] The definition is in fact an assessment of the probability that a fracture will redisplace. Maintaining reduction with acceptable radiological parameters is of essence in treatment of such injuries, we, in our study, have attempted to achieve optimal results with open reduction with volar plating and augmenting with 2–3 K-wires, which were used to maintain alignment and rotation of lateral distal radial and medial radius column. At present, for the management of unstable distal radial fractures, dual plating (volar and styloid plate) or external fixation with K-wire augmentation is used,[5] our method aims to provide less invasive and easily reproducible alternative to dual plating and early mobilization with prevention of complications of stiffness compared to external fixation.

MARERIALS AND METHODS

Forty-eight patients with unstable distal radius fracture were prospectively selected for the study between 2019 and 2021 in tertiary care hospital orthopedic department. Male cases were 27 and 21 cases are female. The mean age of the patients was 51 years. In 36 patients', dominant hand was fractured. All the fractures were unstable comminuted and intra-articular, presenting within 1–3 days of injury. All 48 patients were under regular fallow-up. The common cause of injury was fall on outstretched hand in 36 patients, and 10 cases were due to road traffic accident and remaining two cases the fracture were sustained due to sports-related injury. All are closed fracture and classified according to AO/OTA [Figure 1] using anteroposterior (AP) and lateral view X-rays [Figure 1]. Twenty-two cases were B3, and 18 cases were C1, and eight cases are C2. In addition, radial length, palmer tilt, and radial angulations were measured.

Selection Criteria

Inclusion criteria

- Clinical signs and symptoms
- Radiological findings conforming intra-articular fracture of distal end radius
- Patients who are medically fit and willing for surgery
- Patients between the age groups of 18 and 75 years of both sexes.

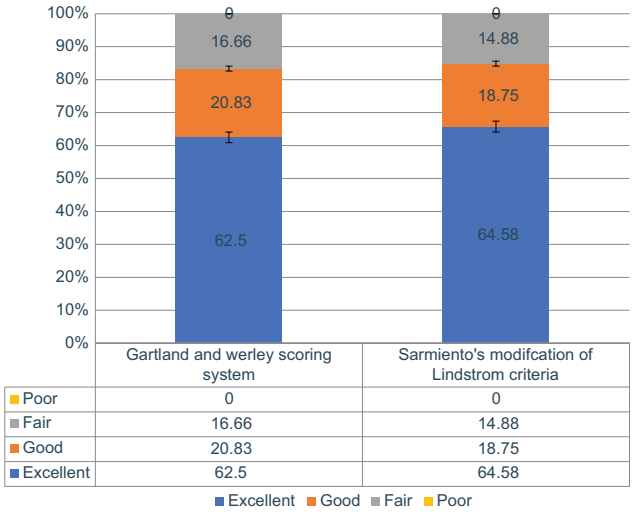
Exclusion criteria

- Patients below 18 years and above 75 years
- Distal end radius fracture associated with other injuries around the wrist joint of same limb
- Pathological fractures
- Distal end radius fracture associated with neurovascular deficit
- There is evidence that the patients will be unable to adhere to trial procedures or complete questionnaires
- Patients with any open wound on same limb.

Patient demographics	
Range of age at time of presentation	21–71 years
Mean age at the presentation	39.15±30.12
Male patients	27
Female patients	21
Fracture subtype B3	22
Fracture subtype C1	18
Fracture subtype C2	8
Right hand dominance	27
Left hand dominance	21
Total number of patients	48

Parameters	Postoperatively at 6 weeks	At 1-year follow-up
Radial inclination	21.06±3.25 degrees	20.96±4.40 degrees
Radial height	10.12±1.48 mm	10.06±1.18 mm
Palmar tilt	9.64±5.84 degree	9.55±5.18 mm
Ulnar variance	−0.28±0.68 mm	−0.12±1.10 mm

Parameters	Postoperatively at 6 weeks	At 1-year follow-up
	In degrees	In degrees
Flexion	40.12±14.15	61.13±12.45
Extension	29.15±7.40	50.65±18.42
Supination	55.10±9.10	77.42±11.38
Pronation	74.10±8.10	84.32±5.30
Grip strength (compared to the opposite side)	56.45±20.45	89.50±4.25



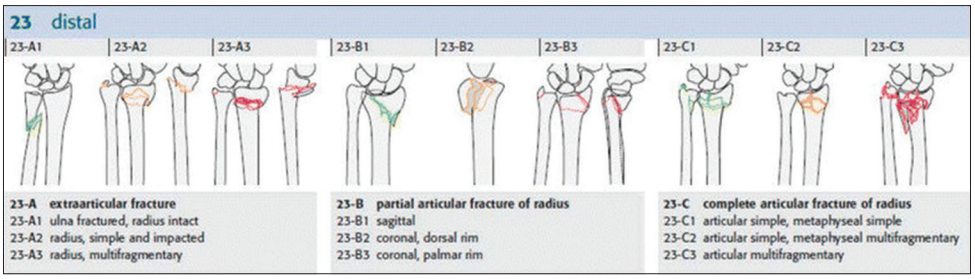


Figure 1: Classification of distal radius fractures

Surgical Approach for Volar Plate Fixation

All surgeries were performed under regional or general anesthesia under fluoroscopic guidance. With the patient positioned on the operating table, the skin incision was made after palpating the flexor carpi radialis tendon (FCR). The sheath of FCR was split and the FCR was retracted ulnar ward. The interval was developed between the FCR tendon and the radial artery. The pronator quadratus (PQ) was exposed, and incision was made over the radial border of the PQ. The fracture site was exposed, and the periosteum was elevated using a periosteal elevator. The fracture was the reduced using a volar and ulnar directed force with traction being applied to maintain reduction, a K-wire was inserted through the fracture site from the radial styloid to stabilize the styloid fragment of fracture and another K-wire from listers tubercle to maintain apposition of the dorsal fracture fragments. An appropriately sized distal radius LCP was selected depending on the fracture pattern. The plate was fixed under the guidance of the image intensifier and the articular reduction was maintained with subchondral screws. At the end of the procedure, the detached end of the PQ was reattached to the edge of brachioradialis to provide coverage for the distal plate. Carpal tunnel was not decompressed in any of our cases. Distal radioulnar joint (DRUJ) instability was assessed in all cases intraoperatively after the fixation of the distal radius by ballottement test, with the forearm in a neutral position. DRUJ instability was suspected if there was a soft endpoint with an increase in the AP translation of 5–10 mm as compared to the uninjured wrist. In such cases, DRUJ was temporarily transfixed with radioulnar K-wire for a period of 4 weeks and the wire was removed in the outpatient department. All the patients were operated through the same approach.

The skin was closed in layers after a thorough wash. A bivalve slab was applied in all cases and was maintained for a period of 3 weeks post-operative. Post-operative radiographs were taken. The patients were then reviewed at post-operative weeks 3, 6, 12, and 24. AP and lateral radiographs were taken during all the reviews, the slab and K-wires were removed at 6 weeks and wrist mobilization in terms of range of motion exercises and soft ball exercises to improve grip strength were started at week 3. During the final review at post-operative week 24, the final scoring of wrist function was done using the Gartland and Warley functional scoring and Sarmiento radiological score. The results were tabulated and analyzed.

Post-operative Management

Postoperatively, the limbs were placed into a bulky dressing, post-surgery active finger movements were encouraged. Suture removal was done on the 12th day. Ulnar deviation, palmer deviation, and active rotational exercise were started 6 weeks postoperatively.

Follow-Up Period

Patients were followed up post-operative visits at 2 and 6 weeks and 3, 6, and 12 months post-surgery. All relevant findings recorded in every visit, functional assessment system and radiological assessment of the fractures progression were done.

	Residual deformity	Loss of radial tilt (°)	Radial shortening (ml)	Loss of radial deviation (°)
Excellent	No deformity/ insignificant	0	<3	5
Good	Slight	1-10	3-6	5-9
Fair	Moderate	11-14	7-11	10-14
Poor	Severe	At least 15	≥12	>14

Sarmiento's modification of Lindstrom criteria.

RESULTS

Forty-eight patients between the age of 21 years and 71 underwent surgery for distal radius fracture. Mean age in patient was 39.15 years. All patients were active and independent. Table 1 shows patient demographics. About 66% of fractures were as a result of a simple fall; 17% in falls from bicycles, 15% in motorbike accidents, and 2 in blunt trauma. All fractures were unstable. Mean operating time was 85 min (51–118 min range). Mean time to surgery was 4.7 days (range 2–14 days). All fractures united between 9 and 17 weeks of surgery (mean 11 weeks). Table 2 shows range of the wrist movements. Mean VAS was 1.6 (range 0–7), mean grip strength was 84.5 (range 55–98). The complication rate was 17.1% [Figures 2-7].

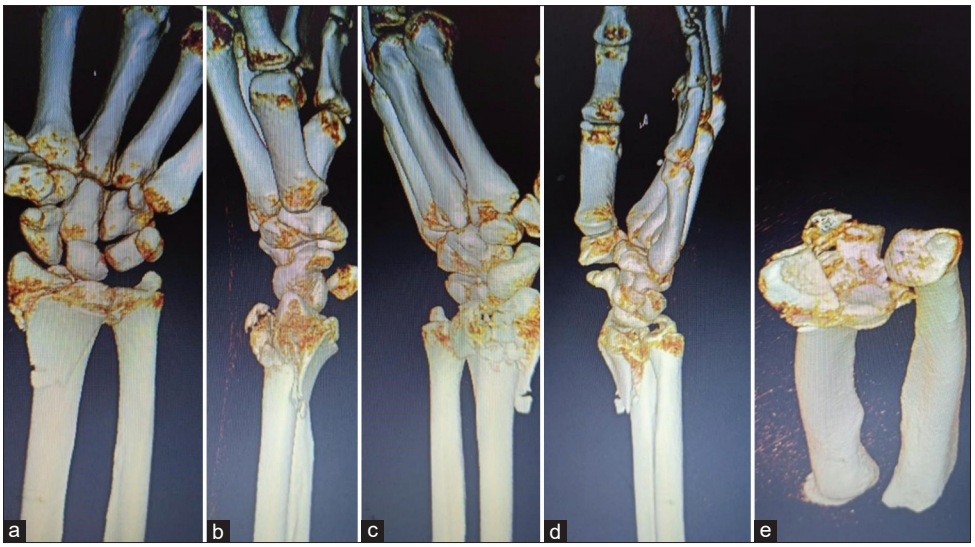


Figure 2: (a-e) Computed tomography showing fracture pattern

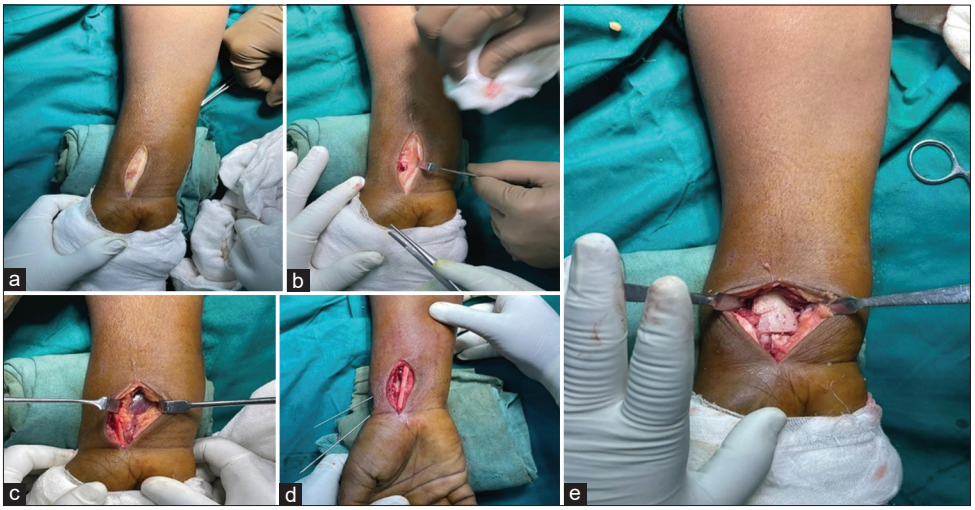


Figure 3: (a-e) Surgical approach and intraoperative images

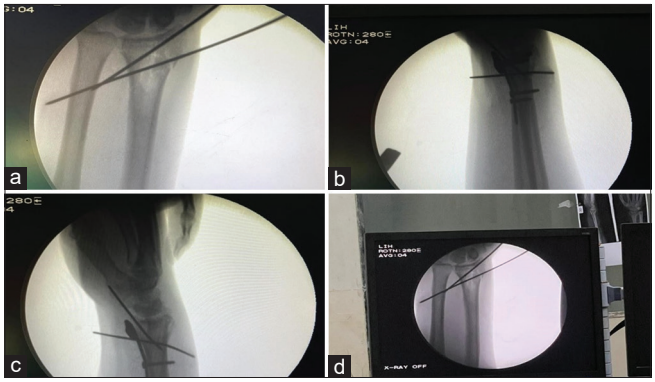


Figure 4: (a-d) C-arm images showing use of K-wire for achieving initial reduction

DISCUSSION

Fracture of the distal radius remains the most common fracture treated by orthopedic trauma surgeons, but despite this, there is no consensus about the treatment of more complex cases with metaphyseal instability or intra-articular displacement.[6] Surgical treatment must address both the intra-articular displacement and any accompanying metaphyseal displacement and instability, so a combination of techniques may be required. Each fracture must be assessed to ascertain the fracture pattern and displacement of the fragments and a treatment strategy defined on this basis.[7] A knowledge of the typical fracture patterns as described by Melone is useful. He described four parts – radial styloid, dorsal, and ulnar volar fragments, and the radial shaft. As the lunate impacts on to the articular surface, the lunate facet may be depressed as one or split into a volar and dorsal component with added central impaction.[8] The impact of the scaphoid on the radial styloid typically causes a shearing fracture. An understanding of this mechanism is helpful to the surgeon in planning procedures.

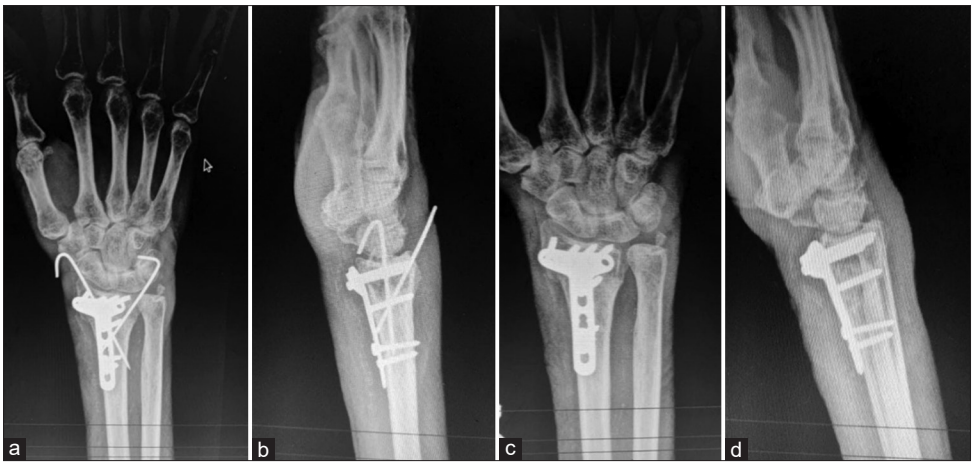


Figure 5: (a-d) Radiographic images showing union at fracture site, removal of K-wires was done when union was visible

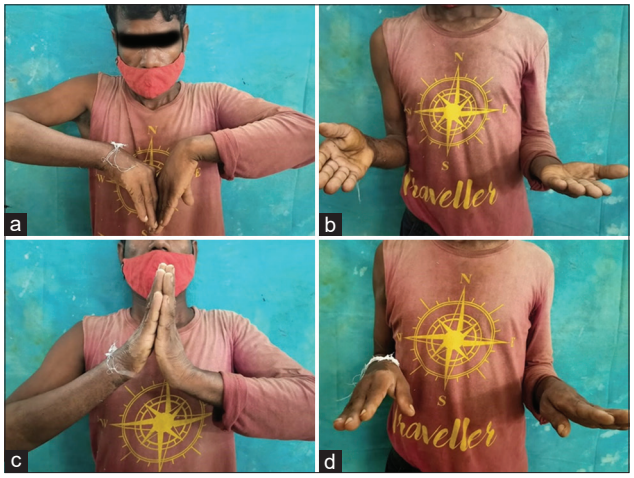


Figure 6: (a-d) Clinical outcome at 6 months

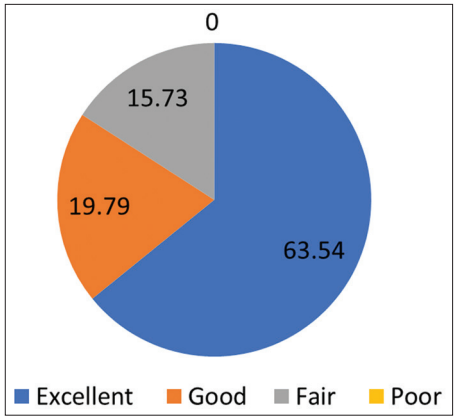


Figure 7: Clinico-radiological outcome

Previous biomechanical studies have suggested that the middle column of the distal radius is primary load-bearing surface, which transmits an axial load to the wrist and has a major role in mechanical conduction.[9] The fracture line of middle column of the distal radius is mostly vertical. The radial column of the distal radius mainly stabilizes the wrist and controls rotation and its fracture line is usually horizontal,[10] so the styloid fracture fragment is prone to rotation, even though a screws from locking plate (LCP) hold and compress the displacement but there is a need for additional support to maintain radial inclination and height, for this conventionally radial styloid plating with volar plating was done, we in our study have used K-wire augmentation to achieve stability.

The goal of treatment is to achieve a painless wrist with a good function. Malunion can result in persistent pain, reduced range of motion, decreased grip power, limitation in day-to-day activities, which can be disabling.[11,12] Various surgical treatment options are available for the treatment of distal radius fractures. Each technique has advantages and disadvantages. Intramedullary nailing for distal radius fractures is not used too often due to a higher complication rate.[13] Closed reduction and K-wiring are less costly but drawbacks are prolonged immobilization, pin-tract infection, stiffness, and complex regional pain syndrome, may not be sufficient in comminuted fractures, and loss of reduction after removal of K-wires. Grip strength and supination is better in patients with unstable distal radius fractures treated with a volar locking than those treated with closed reduction and percutaneous K-wiring only.[14] External fixation once popular is not used widely now due to a variety of reasons that include post-operative stiffness, patient inconvenience, pin-tract infection, pin loosening, loss of reduction following removal, median, and superficial nerve neuropathies.[15,16] External fixation when compared with plating for unstable distal radius fractures has higher complications including infection and malunion. The clinical and radiological results of a volar LCP are superior to a non-bridging and bridging external fixator in treatment of intra-articular comminuted distal radius fractures. Dorsal plating was once popular but not used widely due to late complications and volar collapse when compared with volar LCP and no difference in outcomes. The use of a volar LCP with K-wire augmentation proves beneficial in restoration of the anatomy, stability,

shorter duration of immobilization, early range of motion exercises, and early return to function.

In our method, we used volar plating it allowed direct visualization of fracture fragments, which were reduced and maintained with two or three K-wires, one from radial styloid to hinder its rotation and one or two K-wires from dorsal aspect for maintaining anatomical volar tilt, it aided in restoration of the anatomy, decreased morbidity by allowing early mobilization, and early return of wrist function. LCP s address intra-articular and metaphyseal comminution and are mostly helpful in osteoporotic fractures preventing late collapse of fracture fragments.[17] Biomechanical studies comparing volar fixed angle LCP s with that of conventional dorsal plates report volar fixed-angle plates to be superior in terms of their strength.[18] Dorsal plating of distal radius has lost popularity due the fact that, in spite of dorsal plating, the volar collapse of fracture occurred which resulted in malunion and a second surgery in later period.[19] Complications associated with plating include risk of infection as compared to closed procedures, tendon irritation, or rupture. These may warrant implant removal in some cases.[20,21]

In our study, the dominant side which was injured recovered early, with grip strength 96% of the contralateral side at 6 months follow-up and remained the same at 1 year of follow-up. Grip strength of the non-dominant injured side reached about 84% of the normal side even at 1 year of follow-up. Pronation improved more rapidly than supination at 6 weeks of follow-up but both returned to

Results	Points
Residual deformity	
Prominent ulnar styloid	1
Residual dorsal tilt	2
Radial deviation of hand	2-3
Point range	0-3
Subjective evaluation	
Excellent. No pain, disability, or limitation of motion	0
Good. Occasional pain, slight limitation of motion, no disability	2
Fair. Occasional pain, some limitation of motion, feeling of weakness in wrist, no particular disability if careful, activities slightly restricted	4
Poor. Pain, limitation of motion, disability, activities more or less markedly restricted	6
Point range	0-6
Objective evaluation	
Loss of dorsiflexion	5
Loss of ulnar deviation	3
Loss of supination	2
Loss of palmar flexion	1
Loss of radial deviation	1
Loss of circumduction	1
Pain in distal radio-ulnar joint	1
Point range	0-5
Complications	
Arthritic change	
Minimal	1
Minimal with pain	3
Moderate	2
Moderate with pain	4
Severe	3
Severe with pain	5
Nerve complications (median)	1-3
Poor finger function due to cast	1-2
Point range	0-5
End-result point ranges	
Excellent	0-2
Good	3-8
Fair	9-20
Poor	21 and above

Gartland and Warley demerit scoring system

near normal at the 1 year of assessment. At the end of 1 year follow-up, 63.54% of patients had excellent and 19.79% had good results, 15.73% of patients had fair results, and no one had poor outcome.

CONCLUSION

We conclude that fixation of distal radius fracture with LCP and stabilization of the styloid fracture fragment with K-wire augmentation had a favorable impact on the outcome of the study. For unstable fractures of the distal end of radius, volar LCP osteosynthesis with K-wire augmentation is a valid and effective treatment option ensuring a successful functional and radiological outcome.

CONSENT

Written informed consent was obtained from all the patients for publication of this report and accompanying images.

REFERENCES

1. Johnson NA, Cutler L, Dias JJ, Ullah AS, Wildin CJ, Bhowal B. Complications after volar locking plate fixation of distal radius fractures. Injury 2014;45:528-33.
2. Fok MW, Klausmeyer MA, Fernandez DL, Orbay JL, Bergada AL. Volar plate fixation of intra-articular distal radius fractures: A retrospective study. J Wrist Surg 2013;2:247-54.
3. Arora R, Lutz M, Deml C, Krappinger D, Haug L, Gabl M. A prospective randomized trial comparing non-operative treatment with volar locking plate fixation for displaced and unstable distal radial fractures in patients sixty-five years of age and older. J Bone Joint Surg Am 2011;93:2146-53.
4. Anakwe R, Khan L, Cook R, McEachan J. Locked volar plating for complex distal radius fractures: Patient reported outcomes and satisfaction. J Orthop Surg Res 2010;5:51.
5. Arora R, Gabl M, Gschwentner M, Deml C, Krappinger D, Lutz M. A comparative study of clinical and radiologic outcomes of unstable Colles type distal radius fractures in patients older than 70 years: Nonoperative treatment versus volar locking plating. J Orthop Trauma 2009;23:237-42.
6. Arora R, Lutz M, Hennerbichler A, Krappinger D, Espen D, Gabl M. Complications following internal fixation of unstable distal radius fracture with a palmar locking-plate. J Orthop Trauma 2007;21:316-322.
7. Grala P, Zieliński W. Hybrid external fixation for neglected fractures of the distal radius: Results after one year. J Orthop Traumatol 2008;9:195-200.
8. Grala P, Kierzynka G, Machynska-Bucko Z. Hybrid external fixation of unstable distal radius fractures: Initial experience. J Orthop Traumatol 2005;6:138-44.
9. Rayhack J, Langworthy J, Belsole R. Transulnar percutaneous pinning of displaced distal radial fractures: A preliminary report. J Orthop Trauma 1989;3:107.
10. Nagi ON, Dhillon MS, Aggarwal S, Deogaonkar KJ. External fixators for intra-articular distal radius fractures. Indian J Orthop 2004;38:19-22.
11. Ruch DS, Ginn TA. Open reduction and internal fixation of distal radius fractures. Op Tech Orthop 2000;13:138-43.
12. Doi K, Hattori Y, Otsuka K, Abe Y, Yamamoto H. Intra-articular fractures of the distal aspect of the radius: Arthoscopically assisted reduction compared with open reduction and internal fixation. J Bone Joint Surg Am 1999;81:1093-110.
13. Kim JY, Tae SK. Percutaneous distal radius-ulna pinning of distal radius fractures to prevent settling. J Hand Surg Am 2014;39:1921-5.
14. Ruch DS, Papadonikolakis A. Volar versus dorsal plating in the management of intra-articular distal radius fractures. J Hand Surg 2006;31:9-16.
15. Minegishi H, Dohi O, An S, Sato H. Treatment of unstable distal radius fractures with the volar locking plate. Ups J Med Sci 2011;116:280-4.
16. Jose A, Suranigi SM, Deniese PN, Babu AT, Rengasamy K, Najimudeen S. Unstable distal radius fractures treated by volar locking anatomical plates. J Clin Diagn Res 2017;11:RC04-8.
17. Knudsen R, Bahadirov Z, Damborg F. High rate of complications following volar plating of distal radius fractures. Dan Med J 2014;61:A4906.
18. Young BT, Rayan GM. Outcome following nonoperative treatment of displaced distal radius fractures in low-demand patients older than 60 years. J Hand Surg Am 2000;25:19-28.
19. Beumer A, McQueen MM. Fractures of distal radius in low-demand elderly patients: Closed reduction of no value in 53 of 60 wrists. Acta Orthop Scand 2003;74:98-100.
20. Beharrie AW, Beredjiklian PK, Bozentka DJ. Functional outcomes after open reduction and internal fixation for treatment of displaced distal radius fractures in patients over 60 years of age. J Orthop Trauma 2004;18:680-6.
21. Osada D, Viegas SF, Shah MA, Morris RP, Patterson RM. Comparison of different distal radius dorsal and volar fracture fixation plates: A biomechanical study. J Hand Surg Am 2003;28:94-104.

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Original Article

Determining the Correlation between Ventilatory Function Tests and Heart Rate Variability in Tobacco Smokers of Haryana

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Abstract

Introduction: Tobacco smoking is responsible for 90% of chronic obstructive pulmonary disease. It is also a major risk factor for the development various cardiovascular disorders. It induces autonomic imbalance typically characterized by sympathetic hyperactivity. Extent of airways involvement can be studied by ventilatory function tests. Impact of the nicotine on cardiovascular autonomic functions can be best diagnosed using the heart rate variability (HRV). We intended to seek out any association between these two types of tests. Very few studies are available on correlation between ventilatory function tests and HRV. Hence, this study is conducted.

Materials and Methods: A total of 60 male subjects in the age group of 25–50 years - 30 tobacco chewers and 30 tobacco non users were included. Subjects with history of hypertension, diabetes, oral lesion, drug intake, etc., were excluded from the study. Ventilatory function tests were carried out using RMS Med spirometer. HRV was performed by Polyrite-26D.

Results: A significant negative correlation between $FEF_{25-75\%}$ and mean HR, and $FEF_{25-75\%}$ and LF (ms2) and also between MVV and LF. Apart from this, we got ventilatory function tests' values suggesting obstructive airway changes. HRV parameters suggested increased sympathetic tone in smokers.

Discussion: Smoke and its other constituents cause structural changes in airways leading to obstruction. Nicotine is the main culprit for autonomic disturbances. As far as correlation between the two is concerned, we failed to obtain significant association between the parameters of both tests.

Key words: Correlation, HRV, Ventilatory, Smokers, Tobacco

INTRODUCTION

Tobacco smoking is responsible for 90% of chronic obstructive pulmonary disease. It is also a major risk factor for the development of atherosclerosis, coronary heart disease, acute myocardial infarction, and sudden cardiac death.[1] It also induces autonomic imbalance typically characterized by sympathetic hyperactivity. Impact of the nicotine on cardiovascular autonomic functions can be best diagnosed using the heart rate variability (HRV). Very few studies are available on correlation between ventilatory function tests and HRV. Hence, this study is conducted.

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MATERIALS AND METHODS

The present study was conducted in the Department of Physiology, Pt. B.D. Sharma PGIMS, Rohtak. A total of 90 male subjects of age group 25–50 years were included in the study. The subjects were divided into three groups. Study was carried out after ethical approval from the institutional ethical committee. Informed consent was obtained from the subjects before proceeding with the

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procedure. Information was provided in the language familiar to the subjects.

- Smokers – 30 male volunteers who were chronic tobacco smokers for minimum 10 pack years in continuation with duration of 7 years or more
- Control – 30 male volunteers who had never used tobacco in any form (control group).

Subjects with known history or symptoms of any chronic cardiopulmonary, endocrine, or metabolic disorder. Oral lesion, any drug intake was excluded from the study.

Tests Conducted

1. Ventilatory function tests
2. HRV.

Procedure for Recording Ventilatory Functions

The ventilatory functions were recorded using the RMS Med spirometer. The Med spirometer is an instrument which measures inspiratory and expiratory parameters. The test progress is shown on the computer monitor. The subjects were instructed to apply mouth piece closely to the lips and close their nose with nose clip so as to prevent any leakage of air. Following parameters were recorded:

- Forced expiratory volume in first second (FEV_1)
- Forced vital capacity (FVC)
- FEV_1/FVC%
- Forced expiratory flow rate $_{25\text{-}75\%}$ ($FEF_{25\text{-}75\%}$)
- Maximum voluntary ventilation (MVV)
- Peak expiratory flow rate (PEFR)

Procedure for Recording FEV_1, FVC, Maximal Expiratory Flow Rate $(MEFR)_{25\text{-}75\%,}$ and PEFR

For recording of FVC, FEV_1, $MEFR_{25\text{-}75\%,}$ and PEFR, the subjects were asked to breathe in and out normally into the mouth piece. Then, the subjects were asked to take deep breath to fill lungs to maximum possible and then exhale into the mouth piece as quickly as possible. All the subjects made three such attempts and the best of them was selected.

Procedure for Recording MVV

For recording of MVV, subjects were asked to inhale and exhale as deeply and quickly for 15 s. Then, MVV was calculated in liters/minute. The subjects were instructed to stop if they felt any discomfort. Spirometric indices were calculated using best out of three technically satisfactory performances as per recommendations of American Thoracic society.[2]

Procedure for Recording HRV

For recording HRV, Digitalized Power lab 26T Polyrite D was used. Sampling rate was 256 Hz. High and low filters were set at 99 and 0.1 Hzs, respectively. The screen sweep speed was kept at 30 mm/s. For R wave detector, channel 3, that is, ECG channel 3 was used. The whole channel was selected for HRV analysis. Position of event is taken as maximum after threshold. Retrigger delay is taken as 0. Ectopics are excluded from the analysis.

Method of Measurement

HRV of subjects was measured with digitalized Polyrite D as per standards laid by Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.[3]

Procedure

The subjects were asked to lie down on the couch and made to relax in front of the Polyrite D system. The three disposable adhesive electrodes were attached to the left arm, right leg, and left leg, respectively. The basal recording of ECG (Lead II) was taken for 5 min. From the ECG, the analysis of HRV was done automatically by Fast Fourier Transformation method.

Outcome of Variables

HRV parameters generated and selected for the study.

- Mean heart rate (beats/min)
- Mean RR interval (seconds)
- Very low frequency (VLF) (ms^2)
- Low frequency (LF) (nu)
- High frequency (HF) (nu)
- LF/HF ratio

Statistics

All the data obtained by above two procedures were analyzed by commercially available software package SPSS software. Statistical significance between smokers and controls was determined using student's unpaired *t*-test. Pearson's correlation coefficient (r) was used for correlation purpose. $P < 0.05$ was considered statistically significant and $P < 0.001$ was considered highly significant.

RESULTS

The anthropometric variations such as age, height, weight, and BMI were comparable in both the groups. All the ventilatory parameters were reduced in smokers and the reduction was highly significant except for PEFR [Table 1]. Among HRV parameters, there was mean reduction in all the parameters except RR interval and LF/HF ratio. The reduction was significant for HF. A highly significant increase in VLF and LF/HF ratio was also seen [Table 2].

Pearson's correlation between ventilatory function tests and HRV parameters smokers group shows a significant negative correlation between $FEF_{25\text{-}75\%}$ and mean HR, and $FEF_{25\text{-}75\%}$ and LF (ms^2). The negative correlation between MVV and LF is also significant. Correlation coefficient

Table 1: Ventilatory Function Tests in smokers and controls

Parameters	Smokers (n=30) (Mean ± SD#)	Controls (n=30) (Mean ± SD)	p value
FEV_1 (litres)	1.51±.64	2.87±.41	0001**
FVC (litres)	1.86±0.66	3.17±.52	0001**
FEV_1/FVC (%)	80.8±15.2	96.24±11.0	0001**
$FEF_{25-75\%}$ (litres/second)	1.85±1.24	3.96±1.06	0001**
MVV (litres/minute)	68.1±32.5	125.9±25.7	0001**
PEFR (litres/minute)	3.36±1.97	7.05±1.98	9784

*p <0.05 = significant
**p<0.001 = highly significant
SD= Standard deviation

Table: 2 Comparison of HRV parameters in smokers and controls

Parameters	Smokers (n=30) (Mean ± SD#)	Controls (n=30) (Mean ± SD)	*p* value
HR(beats/minute)	84.48±25.95	73.94±15.0	0.074
RR interval(seconds)	747±0.144	760±93.38	0.687
VLF (ms^2)	443.79±253.85	1977.13±1104.22	0.0001**
LF (nu)	58.10±41.77	59.91±12.87	0.861
HF (nu)	17.76±11.39	47.07±63.16	0.014*
LF/HF	3.37±0.61	2.02±0.92	0.0001**

*p <0.05 = significant
**p<0.001 = highly significant
SD= Standard deviation

among other parameters is very weak and statistically insignificant.

DISCUSSION

The findings of ventilatory function tests in our study suggested obstructive changes in the airways. This finding is similar to other studies.[4-6] Smoking is responsible for 90% of the obstructive diseases. After 20 years of smoking, pathophysiologic changes in the lungs develop and progress proportional to smoking intensity and duration. Chronic mucous hyperplasia of the larger airways results in a chronic productive cough in as many as 80% of smokers >60 years. Chronic inflammation and narrowing of the small airways and enzymatic digestion of alveolar walls resulting in pulmonary emphysema can result in reduced expiratory airflow sufficient to produce clinical symptoms of respiratory limitation in 15–25% of smokers.[1]

Most of the HRV parameters were also significantly reduced in smokers' group. However, there was highly significant increase in LF/HF ratio suggesting sympatho-vagal imbalance. Smoking acutely reduces baseline levels of vagal-cardiac nerve activity and completely resets vagally mediated arterial baroreceptor-cardiac reflex responses.[7]

Table 3: Pearsons Correlation between Ventilatory Function Tests and HRV parameters in smokers

Variables	Mean HR	Mean RR	VLF (ms^2)	LF (nu)	HF (nu)	LF/HF
FEV_1	-0.290	0.080	0.239	-0.446	-0.221	-0.247
FVC	-0.227	0.023	0.242	-0.313	0.159	-0.208
FEV_1/FVC	-0.192	0.155	0.177	-0.287	0.240	-0.188
$FEF_{25-75\%}$	-0.325*	0.216	0.278	-0.364*	0.126	-0.197
MVV	-0.263	0.126	0.162	-0.336*	0.063	-0.235
PEFR	-0.314	0.275	0.285	-0.261	-0.091	-0.048

*p <0.05 = significant

These effects are attributed mainly to the action of nicotine that binds to nicotinic cholinergic receptors present in the autonomic ganglia, neuromuscular junctions, and central nervous system, which on stimulation, increases the release of several neurotransmitters.[8] The nicotine and others substances found in cigarettes also stimulate the release of adrenalin into the sympathetic nervous system. In addition, the stimulation of the nicotinic receptors in the autonomic nervous system has been associated with the sympathetic excitatory effects of smoking.[9]

The respiratory cycle also affects autonomic control. The heart rate increases during inspiration and decreases during expiration, causing fluctuations in HRV.[10,11] This physiological phenomenon is known as respiratory sinus arrhythmia. However, we could not find correlation between all the parameters of ventilatory function tests and HRV. A significant negative correlation was seen between $FEF_{25-75\%,}$ mean HR, and Fand LF and between MVV and LF. Based on these findings, we can assume that pulmonary function is poorly associated with autonomic control. However, very few studies are available on it, so this domain needs further exploration.

CONCLUSION

It is well known fact that tobacco smoking leads to obstructive changes in the respiratory tract. Smoking also alters the sympatho-vagal imbalance by increasing sympathetic activity. However, as far as correlation between ventilatory function tests and heart rate variability in smokers is concerned, we could not find significant association except for very few parameters. Since, limited studies are available on corelation between these two tests, further research is required to explore this domain.

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REFERENCES

1. Hecht S. Carcinogen derived biomarkers: Applications in studies of human exposure to secondhand tobacco smoke. Tob Control 2004;13 Suppl 1:i48-56.
2. Brusasco V, Gapo R, Viegi G. Standardization of spirometry. Series ATS/ERS task force: Standardization of lung function testing. Eur Respir J 2005;26:319-38.
3. Malik M, Bigger JT, Camm AJ, Kleiger RE, Mallini A, Moss AJ, *et al*. Task force of the European society of cardiology and the north American society of pacing and electrophysiology: Heart rate variability standards of measurement, physiological interpretation and clinical use. Euro Heart J 1996;17:354-81.
4. Bano R, Mahagaonkar AM, Kulkarni NB, Ahmed N, Nighute S. Study of pulmonary functions among smokers and non-smokers in a rural area. Pravara Med Rev 2009;1:11-6.
5. Nighute S, Tiwari A. A study of the pulmonary function tests among smokers and non-smokers in a rural area of Gujarat. J Clin Diagn Res 2011;5:1151-3.
6. Behera J, Sood S, Gupta R, Kumar N, Singh M, Gupta A, *et al*. Assessing autonomic function in smokers. Australas Med J 2010;3:712-5.
7. Niedermaier ON, Smith ML, Beightol LA, Zukowska-Grojec Z, Goldstein DS, Eckberg DL, *et al*. Influence of cigarette smoking on human autonomic function. Circulation 1993;88:562-71.
8. Shinozaki N, Yuasa T, Takata S. Cigarette smoking augments sympathetic nerve activity in patients with coronary heart disease. Int Heart J 2008;49:261-72.
9. Adamopoulos D, van de Borne P, Argacha JF. New insights into the sympathetic, endothelial and coronary effects of nicotine. Clin Exp Pharmacol Physiol 2008;35:458-63.
10. Grossman P, Wilhelm FH, Spoerle M. Respiratory sinus arrhythmia, cardiac vagal control, and daily activity. Am J Physiol Heart Circ Physiol 2004;287:H728-34.
11. Bianchim MS, Sperandio EF, Martinhão GS, Matheus AC, Lauria VT, da Silva RP, *et al*. Correlation between heart rate variability and pulmonary function adjusted by confounding factors in healthy adults. Braz J Med Biol Res 2016;49:e4435.

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Original Article

Diagnostic Role of Neutrophil-Lymphocyte Ratio in Oral Cavity Cancers: A Prospective Study

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Abstract

Introduction: Squamous cell carcinoma (SCC) of oral cavity and oropharyngeal region is considered as an aggressive malignant neoplasm, because of high morbidity and mortality. It commonly occurs in middle-aged males and older individuals.

Aims and Objectives: The purpose of the study was to analyze the relationship between preoperative levels of neutrophil-lymphocyte ratio (NLR) with clinicopathologic factors and prognosis in oral cavity SCC patients.

Materials and Methods: A total of 810 subjects were studied from January 2019 to December 2020 and 270 each were in three different groups – malignant, premalignant, and control group. Inclusion criteria were to be available with common blood count just before procedure. The hemogram parameters including NLR were compared between the groups.

Results and Conclusion: Lymphocyte count was found to be significantly decreased in oral cavity cancers (OCCs) compared with premalignant oral cavity lesions and control group. In contrast, NLR was significantly higher in OCCs and in oral SCC compared with premalignant oral cavity lesions and control group. The receiver operating characteristics curve analysis suggested of 2.75 as NLR cutoff value in predicting malignancy. Area under curve denotes 0.973, sensitivity - 91.11%, specificity - 91.48%. NLR was first shown to be significantly elevated in OCCs and in oral cavity SCC in this study. In our opinion, NLR may be helpful in identifying high-risk lesions of oral cavity harboring malignancy. It, therefore, has significant potential as a biomarker for risk stratification in oral cavity SCC.

Key words: Leukocyte, Neutrophil-lymphocyte ratio, Oral cavity cancer, Squamous cell carcinoma

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is one of the most common head-and-neck cancers and is the 8th most common cancer worldwide.[1] In India, OSCC ranks number one in terms of incidence among men and third among women.[2] In majority of SCCs, the patient had a history of tobacco chewing and is preceded by potentially malignant lesions, such as leukoplakia, erythroplakia, and oral submucous fibrosis (OSMF). The possible precancerous nature of submucous fibrosis was first mentioned by Paymaster in 1956 who described the development of slow-growing SCC in one-third of the cases with submucous fibrosis.[3]

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Carcinoma development is an intricate complex mechanism and the multifactorial causation makes it more difficult to find specific prognostic and therapeutic biomarkers. Thus, the development of newer diagnostic and predictive approaches that are less invasive, economical, and amenable to repeated sampling is imperative.[4] Early detection of OSCC and OSMF transforming into malignancy can drastically improve the treatment outcomes and prognosis.

Attempts have been made at several diagnostic tests which can guide the difference between benign and malignant lesions, one such test being fluorescence

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visualization.[5] Another such technique is narrowband imaging. Such facilities are only available at higher centers where adequate equipment and the expertise are available.[6]

Recently, there is an increasing interest in more basic investigations like the neutrophil-lymphocyte ratio (NLR) as inflammatory responses play diverse roles at different stages of tumor development, including initiation, promotion, malignant conversion, invasion, and metastasis.[7]

SCC of oral cavity and oropharyngeal region is considered as an aggressive malignant neoplasm, because of high morbidity and mortality. Tumorigenesis and tumor development are related not only to the biological characteristics of tumors but also to elements of inflammation in the tumor microenvironment. Many inflammatory factors, such as interleukin-6 (IL-6), IL-1β, and tumor necrosis factor-α, are involved.[8]

NLR is one of the most studied and confirmed prognostic parameters in laryngeal analysis also. NLR is an inexpensive, reproducible, and widely available blood test, and could be a useful as inflammatory marker.[9] Several studies have suggested association between inflammation and cancer. Inflammation plays a dual role in progression and development of cancer. Tumors are infiltrated by leukocytes. Neutrophils help in growth and progression of malignancy, whereas lymphocytic response against tumor cells helps to control tumor growth and progression.[10] Lymphocytes and neutrophils seem to have reverse effects on cancer cell growth and progression.[11] Neutrophils are the most common cell type seen in the early stages of acute inflammation, whereas lymphocytes are enrolled in chronic infections. Systemic inflammation activated by cancer cells is known to be involved in tumor growth by promoting angiogenesis.[5,11]

An elevated NLR can guide the health care worker at a peripheral center to be more mindful of the possibility of a malignant lesion and thus be quick at referring the case for a biopsy. The present study aims to validate the use of this simple biochemical test for the diagnosis of this disease, namely, oral cavity malignancy which carries such a high rate of morbidity and mortality.

MATERIALS AND METHODS

Study Universe

Study was conducted in the outpatient department (OPD) patients attending the Department of Otorhinolaryngology and Head-Neck Surgery, SMS Medical College and Hospital, Jaipur.

Study Place

The present study was conducted on outdoor patients in the Department of Otorhinolaryngology and Head- Neck Surgery, SMS Medical College and Hospital, Jaipur.

Study Period

The study period was from January 2019 to December 2020.

Study Design

This was a cross-sectional.

Study Type

This was a hospital-based descriptive study.

Methodology

The study subjects were divided into three groups as follows:

Group I: 270 patients presenting with histopathologically confirmed SCC of oral cavity.

Histopathologically, oral cavity malignancies were divided on the basis of their degree of differentiation: (1) Well-differentiated SCC (WDSCC), (2) moderately differentiated SCC (MDSCC), and (3) poorly differentiated SCC (PDSCC).

Group II: 270 patients presenting with histologically confirmed pre-malignant lesions.

Group III: 270 control group comprised age- and sex-matched healthy subjects who had no complaint or history of any major illness in recent past and belonged to the similar socioeconomic group as head-and-neck cancer patients.

All the patients who were willing to undergo examinations, blood investigations, and biopsy and were histologically proved were included in the study population. Cases who presented with recurrence, acute, and chronic systemic infection and who underwent prior treatment such as surgery, chemotherapy, and radiation were excluded from the study group.

OBSERVATIONS AND RESULTS

Sample size was calculated at 80% study power and alfa error of 0.05, assuming standard deviation 1.3. For minimum detectable mean difference of NLR 0.35 in oral cavity cancers (OCCs), 262 patients were required in each group as sample size, which was further enhanced and rounded 270 patients in each group as final sample size. Blood sample was subjected to the assessment of common blood count, ALC, and NLR, in healthy

subjects, premalignant cases and malignant cases of OCCs.

In total 270 malignant cases (Group I), mean age was 48.19 years with standard deviation ±12.35 years, while in premalignant group (Group II), mean age was 44.99 years with standard deviation ±12.31. While in control group (Group III), mean age was 35.5 years with standard deviation ±10.56.

In Group I, 23.7% were female and 76.3% were male. This shows male predominance. While in Group II, out of 270 cases, 37% were female and 63% were male. While in Group III, out of 270 cases, 51.1% were female and 48.9% were male. In 270 malignant cases, 78.9% were tobacco chewer and 21.1% were non-chewer. While in group of premalignant, non-chewers were 45.6% and tobacco chewers were 54.4%. While in control group, non-chewers were 73% and tobacco chewers were 27%.

Mean NLR in malignant group was 3.56 ± 1.05, mean NLR in premalignant group was 2.21 ± 0.40 while mean NLR in control group was 1.98 ± 0.38. Mean NLR in MDSCC subtype was 3.69 ± 0.98, was 5.37 ± 1.44 in PDSCC, and was 3.14 ± 0.56 in WDSCC.

Mean NLR was found to be highest in T3 cases which was 4.51, followed by T2 cases 3.69, followed by T1 cases 3.11, and followed by T4 case which was 2.7. From the receiver operating characteristic (ROC) curve, a cutoff value of 2.75 was defined as pre-treatment NLR (sensitivity – 91.11%, specificity – 91.48%, and corresponding area under curve – 0.973) for predicting malignancy [Tables 1-4 and Figure 1].

DISCUSSION

NLR revealed significantly higher values in oral cavity SCC compared with premalignant oral cavity lesions and healthy individuals. It is used as a marker of subclinical inflammation and its higher values are an independent predictor of poor prognosis of various cancers.[12] In head-and-neck malignancies, NLR was first studied in nasopharyngeal carcinoma. Elevated NLR was shown be associated with poor survival in nasopharyngeal carcinoma.[13] Later on, other reports were published regarding the association of elevated NLR for laryngeal SCC diagnosis and survival.[14,15] In the study of Perisanidis *et al.*,[16] elevated NLR was shown to be associated with poor survival in patients with oral cavity cancer who were given pre-operative chemoradiotherapy. The age group of patients included in our study in malignant group varied between 21 years and 79 years with mean age 48.19 years. In premalignant group, it was 20–78 years with mean being 44.99 years whereas the overall mean age of all the cases in the study group was 42.89 years. This was found similar to the study conducted by Perisanidis *et al.* with mean age 48.19 ± 10.21.[16]

Our study showed that majority of cases was male in both malignant and premalignant groups. This was similar to the study conducted by Duzlu *et al.*[15] and Perisanidis *et al.*,[16] this is because of a larger population of males consuming tobacco in a chewable form in Indian subcontinent and hence greater incidence among men.

Majority of patients were tobacco chewers (73%) and 27% were non-tobacco chewers. Our study was similar to a study done by Perisanidis *et al.*,[16] Farrag *et al.*,[17] and Tu *et al.*[18] There were significant associations between oral cancer and tobacco chewing. From the study, we concluded that the mean NLR in malignant group was 3.56 ± 1.05 and in premalignant group was 2.21 ± 0.40, also the cutoff value of NLR in control group was 1.98 ± 0.38. This was similar to Duzlu *et al.*,[15] mean NLR for malignant group was 3.07 ± 1.30, and it was 3.46 ± 1.51 from study of Kum *et al.*[14]

In this study, mean NLR level increases as grading of malignancy goes from well differentiated (3.14) to moderately differentiated (3.69) to poorly differentiated subtype (5.37). Furthermore, the majority of the cases fell in well-differentiated category (48% of all cases) followed by MDSCC (42% of all cases) and PDSCC (10% of all cases). The studies by Farrag *et al.*[17] and Perisanidis *et al.*[16] also demonstrated higher NLR in MDSCC and PDSCC.

In this study showed that with increasing tumor size (staging), the value of NLR increases progressively. Majority of our cases fall into T2 stage (70% of total cases) with mean NLR 3.69 ± 1.11 followed by T1 stage

Table 1: Demographic and clinical characteristics of the study population

Age (in years)							
Group	***n***	**Mean**	**SD**	**Median**	**Minimum**	**Maximum**	***P*-value**
Control	270	35.50	10.56	34.00	17	64	<0.001
Malignant	270	48.19	12.35	45.50	21	79	
Pre malignant	270	44.99	12.31	44.00	20	78	
Total	810	42.89	12.93	42.00	17	79	

Sex	**Group**					
	Control		**Malignant**		**Premalignant**	
	n	**%**	***n***	**%**	***n***	**%**
F	140	51.9	64	23.7	100	37.0
M	130	48.1	206	76.3	170	63.0
Total	270	100.0	270	100.0	270	100.0

Table 2: Comparison of NLR between control, premalignant, and malignant cases of oral cavity

				N: L			
Group	*n*	Mean	SD	Median	Minimum	Maximum	*P*-value
Control	270	1.98	0.38	1.90	0.90	3.10	<0.001
Malignant	270	3.56	1.05	3.30	2.10	8.20	
Premalignant	270	2.21	0.40	2.10	1.20	3.00	
Total	810	2.58	0.98	2.30	0.90	8.20	

NLR: Neutrophil-lymphocyte ratio

Table 3: Correlation of NLR with different histological subtypes of oral cavity cancers

				N: L			
HPR	N	Mean	SD	Median	Minimum	Maximum	*P*-value
MDSCC	115	3.69	0.98	3.60	2.10	8.20	<0.001
PDSCC	23	5.37	1.44	5.00	3.40	8.20	
WDSCC	132	3.14	0.56	3.00	2.10	7.50	
Total	270	3.56	1.05	3.30	2.10	8.20	

NLR: Neutrophil-lymphocyte ratio, WDSCC: Well-differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma, PDSCC: Poorly differentiated squamous cell carcinoma

Table 4: Comparison of NLR between different TNM staging

TNM staging	N: L
T1	
n	71
Mean	3.11
SD	0.49
T2	
n	188
Mean	3.69
SD	1.11
T3	
n	10
Mean	4.51
SD	1.6
T4	
n	1
Mean	2.7
SD	
P-value	<0.001

NLR: Neutrophil-lymphocyte ratio

(26%of total cases) with mean NLR 3.11 ± 0.49 and T3 (3.8% of all cases) with mean NLR 4.51 ± 1.6. These increased progressively from T1 to T3. While in T4 stage, only 1 case (0.3% of total cases) was seen. Mean NLR was 2.7 in T4 stage which was less than T3 stage which might be by chance as there was only one case of T4 lesion in our random study. This was similar to the study conducted by Perisanidis *et al.*[16] –and Fang *et al.*[5] A coexistence of higher NLR had significant association with pathologic tumor status, Farrag *et al.*[17] and Liu *et al.*[19]

Overall, an elevated NLR may be either the result of an excessive but ineffective immune response to the tumor load,

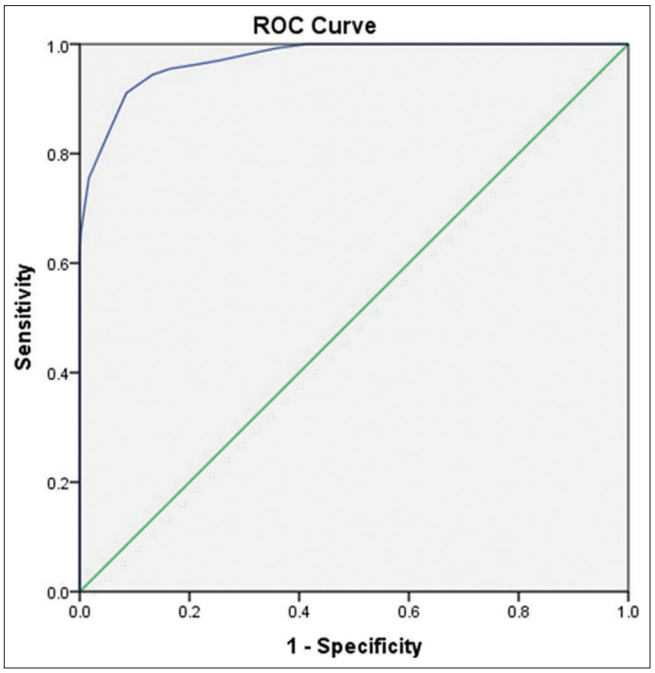

Figure 1: Receiver operating characteristic curve, neutrophil-lymphocyte ratio to predicting malignancy

or it may be a marker of imbalanced inflammatory state which facilitates tumor growth. The ROCs curve analysis suggested cutoff value of 2.75 for NLR in predicting malignancy. In the study by Duzlu *et al.*,[15] the ROC curve analysis suggested cutoff value of 2.88 for NLR in predicting malignancy.

There are several explanations for the relation between elevated NLR and poor prognosis in cancer. The first is that neutrophilia promotes tumor growth. Circulating neutrophils contain and secrete various cytokines, including circulating vascular endothelial growth factor,[20] platelet-derived growth factor, fibroblast growth factor, CXCL8,[21] matrix metalloproteinases,[22] and elastases.[23]

SUMMARY AND CONCLUSION

Inflammation has been shown to play a major role in the development of premalignant lesions and their progression to malignancy. We recommend that hematological investigations like NLR should be routinely evaluated and should be part of standard investigations of oral cavity malignancy, which may help in screening and early diagnosis of oral cavity cancers and their managements.

COMPLIANCE WITH ETHICAL STANDARDS

Consent

written and informed consent taken from all participants.

Ethical approval

Taken from institute ethics committee. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

REFERENCES

1. Balkwill F, Mantovani A. Inflammation and cancer: Back to Virchow? Lancet 2001;357:539-45.
2. Ray JG, Ganguly M, Rao BS, Mukherjee S, Mahato B, Chaudhuri K. Clinico-epidemiological profile of oral potentially malignant and malignant conditions among areca nut, tobacco and alcohol users in Eastern India: A hospital based study. J Oral Maxillofac Pathol 2013;17:45-50.
3. Pundir S, Saxena S, Aggrawal P. Oral submucous fibrosis a disease with malignant potential-report of two cases. J Clin Exp Dent 2010;2:215-8.
4. Gosavi SR, Torkadi AA. Serum C-reactive protein in oral submucous fibrosis and oral squamous cell carcinoma: A cross-sectional study. J Oral Maxillofac Pathol 2020;24:46-51.
5. Fang HY, Huang XY, Chien HT, Chang JT, Liao CT, Huang JJ, *et al*. Refining the role of preoperative C-reactive protein by neutrophil/lymphocyte ratio in oral cavity squamous cell carcinoma. Laryngoscope 2013;123:2690-9.
6. Badakhshi H, Kaul D, Zhao KL. Association between the inflammatory biomarker, C-reactive protein, and the response to radiochemotherapy in patients with esophageal cancer. Mol Clin Oncol 2016;4:643-7.
7. Yodying H, Matsuda A, Miyashita M, Matsumoto S, Sakurazawa N, Yamada M, *et al*. Prognostic significance of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in oncologic outcomes of esophageal cancer: A systematic review and meta-analysis. Ann Surg Oncol 2016;23:646-54.
8. Zhao QT, Yang Y, Xu S, Zhang XP, Wang HE, Zhang H, *et al*. Prognostic role of neutrophil to lymphocyte ratio in lung cancers: A meta-analysis including 7, 054 patients. Onco Targets Ther 2015;8:2731-8.
9. Amirchaghmaghi M, Mohtasham N, Delavarian Z, Shakeri MT, Hatami M, Mozafari PM. The diagnostic value of the native fluorescence visualization device for early detection of premalignant/malignant lesions of the oral cavity. Photodiagnosis Photodyn Ther 2018;21:19-27.
10. Galdiero MR, Varricchi G, Loffredo S, Mantovani A, Marone G. Roles of neutrophils in cancer growth and progression. J Leukoc Biol 2018;103:457-64.
11. Ruegg C. Leukocytes, inflammation, and angiogenesis in cancer: Fatal attractions. J Leukoc Biol 2006;80:682-4.
12. Tao Y, Shi M, Chommanard C, Queen K, Zhang J, Markotter W, *et al*. Surveillance of bat coronaviruses in Kenya identifies relatives of human Coronaviruses NL63 and 229E and their recombination history. J Virol 2017;91:e1916-53.
13. An X, Ding PR, Wang FH, Jiang WQ, Li YH. Elevated neutrophil to lymphocyte ratio predicts poor prognosis in nasopharyngeal carcinoma. Tumour Biol 2011;32:317-24.
14. Kum RO, Ozcan M, Baklaci D, Kum NY, Yilmaz YF, Gungor V, *et al.* Elevated neutrophil-to-lymphocyte ratio in squamous cell carcinoma of larynx compared to benign and precancerous laryngeal lesions. Asian Pac J Cancer Prev 2014;15:7351-5.
15. Duzlu M, Karamert R, Tutar H, Karaloglu F, Sahin M, Cevizci R. Neutrophil-lymphocyte ratio findings and larynx carcinoma: A preliminary study in Turkey. Asian Pac J Cancer Prev 2015;16:351-4.
16. Perisanidis C, Kornek G, Pöschl PW, Holzinger D, Pirklbauer K, Schopper C, *et al.* High neutrophil-to-lymphocyte ratio is an independent marker of poor disease-specific survival in patients with oral cancer. Med Oncol 2013;30:334.
17. Farrag A, Shaukat A, Ali M, Kandil M. Pretreatment neutrophil-to-lymphocyte ratio (NLR) as a prognostic factor of outcome for patients with oral cavity squamous cell carcinoma: A single institution experience. Int J Cancer Clin Res 2018;5:100.
18. Tu XP, Qiu QH, Chen LS, Luo XN, Lu ZM, Zhang SY, *et al*. Preoperative neutrophil-to-lymphocyte ratio is an independent prognostic marker in patients with laryngeal squamous cell carcinoma. BMC Cancer 2015;15:743.
19. Liu CL, Lee JJ, Liu TP, Chang YC, Hsu YC, Cheng SP Blood neutrophil-to-lymphocyte ratio correlates with tumor size in patients with differentiated thyroid cancer. J Surg Oncol 2013;107:493-7.
20. Di Carlo E, Forni G, Musiani P. Neutrophils in the antitumoral immune response. Chem Immunol Allergy 2003;83:182-203.
21. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: Links to genetic instability. Carcinogenesis 2009;30:1073-81.
22. Shamamian P, Schwartz JD, Pocock BJ, Monea S, Whiting D, Marcus SG, *et al*. Activation of progelatinase A (MMP-2) by neutrophil elastase, cathepsin G, and proteinase-3: A role for inflammatory cells in tumor invasion and angiogenesis. J Cell Physiol 2001;189:197-206.
23. Scapini P, Nesi L, Morini M, Tanghetti E, Belleri M, Noonan DM, *et al*. Generation of biologically active angiostatin kringle 1-3 by activated human neutrophils. J Immunol 2002;168:5798-804.

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Original Article

Pattern of Uterine Cervical Smears using Modified Hematoxylin and Eosin Stain in Pregnant Women Attending STD Clinic at a Tertiary Teaching Institute

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Abstract

Two hundred and sixty pregnant women both married and unmarried attending STD clinic at IOV, Madras Medical College with or without genitourinary symptoms were screened for STIs and underwent uterine cervical cytology stained by modified H and E stain. Inflammatory (acute and chronic) changes were noted in majority (75%) with or without clinical cervicitis followed by neoplastic changes in significant numbers, especially in HSV and HPV infections. We advise early intervention by repeat cytology and regular follow-up for those with abnormal smears (chronic inflammatory and intraepithelial neoplastic) to prevent reproductive morbidity and mortality. Only if a repeat cytology is abnormal, postpartum colposcopy and colposcopically directed biopsies will be highly useful to detect severe dysplasia and early carcinomatous changes.

Key words: Pregnancy, Cervical smear cytology, Sexually transmitted diseases

INTRODUCTION

Sexually transmitted diseases (STDs) affecting mankind since antiquity are an important cause of morbidity and mortality worldwide, especially in woman and children, particularly in resource poor settings of developing world, which are store houses of most of the pathogens causing STDs.[1-3]

Infections in pregnancy are common but few cause fetal infection and damage.[4] Pregnancy is a vulnerable time of women.[5] Hence, STDs in the pregnant women are more serious than in the non-pregnant women.[6] Physiological changes, anatomical changes in genital tract, and immunological alterations (alterations in host defense mechanisms) in a pregnant female have been postulated to influence the course of STDs which pose a special risk of infection for both mother and fetus.[7,8]

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Diagnosing sexually transmitted infections (STIs) in pregnancy can be difficult because most are either asymptomatic or too subtle to recognize clinically as pregnancy modifies the manifestations. The cause for screening and treating for asymptomatic genital infectious disease may be stronger during pregnancy than at other times. Adequate treatment must automatically follow as screening is instituted. Study of the prevalence of pattern of cervical smears with or without STDs is important to know to devise appropriate control measures.[9] Antenatal attendees due to their initial entry and later regular reviews in a reproductive health care settings form an important section of population and are commonly used as a reference point for STD prevalence / early screening for cervical carcinoma in the general population of women.[10]

Cervical Screening for Genital Infections in Pregnancy

In recent decades, screening for genital infections has been an integral part of antenatal care.[11] Wet-fixed

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Papanicolaou-stained preparations are traditional techniques for gynecologic cytology. The components of Papanicolaou stain are hematoxylin (nuclear stain), orange G, and eosin (cytoplasmic stains). Hematoxylin and eosin (H and E) stain is occasionally used on wet-fixed material as a rapid stain, but lacks of advantages of Papanicolaou stain for differential staining of cytoplasmic elements.[12] The squamocolumnar junction from where the cervical smear should be taken is at the external os and therefore easily sampled in young and pregnant women.[13,14]

A cervical smear which is taken properly should include squamous epithelial cells, a few neutrophil polymorphs, histiocytes, endocervical or squamous metaplastic cells, and mucin.[13] The smear during early stages shows gradually increasing numbers of intermediate cells and may show cytolysis. Large numbers of navicular cells (intermediate cells that accumulate intracytoplasmic glycogen with thick cells membranes) are often noted.[13,15]

Degenerative changes of epithelial cells are common in a cervical smear taken during acute phase of cervicitis.[13,16] The mere presence of a few neutrophil polymorphs in a smear does not signify inflammation. Large numbers of neutrophils are associated often with an inflammatory process. They may be not be associated with a specific organism (*Neisseria gonorrhoeae*, *Chlamydia trachomatis*, herpes simplex virus (HSV), HPV, *Trichomonas vaginalis*, *Candida* spp., etc.,).[13,16,17] Inflammatory smears are also reported to be associated with bacterial vaginosis (BV) although rare.[17] A bacterial background may be present in association with inflammatory cellular changes, but specific bacteria cannot be reliably identified by cytology alone.[13] Cervical inflammation caused by STI is suspected to promote cervical intraepithelial neoplasia (CIN).[15,18] In chronic cervicitis, inflammatory infiltrate is composed of lymphocytes, plasma cells, and histiocytes. In the presence of cervicitis, the cervix may have red, eroded (cervical erosion') area around the external cervical os.[16] About 40–80% of women with an inflammatory smear have an infection that needs to be treated. Inflammation can accompany CIN.[15]

Candida infection is a common variety seen in cervical smears. It is seen in the form of pink staining pseudohyphae and spores.[13,15] The epithelial cells in the background usually exhibit severe inflammatory changes. Pap smear is unreliable as a diagnostic modality, being positive in only about 25% of culture-positive symptomatic vulvovaginal candidiasis.[7]

Trichomonads are sometimes difficult to identify in smears, since the organisms appear as small ill-defined blobs in the background and they are rounded, oval or pear shaped with the "Mongol eye" nucleus and red cytoplasmic granules.[13] The smear also shows degenerative epithelial changes, leukocytic infiltrate, and an acidophilic reaction in the presence of trichomonads, staining pink irrespective of their maturity.[13,16] Minor epithelial atypia may be associated with trichomoniasis.[16] Using stained cervical smear preparation to diagnose *T. vaginalis* infection is time consuming and cumbersome as compared with vaginal stained smears/wet mounts. The false-negative rate is 30% with Papanicolaou stain.[19,20] *Gardnerella* infection is represented in cervical smear by the clue cell.[13] Gynecologically, BV has been associated with CIN.[21]

HSV type II is believed to be an initiator in the development of carcinoma cervix. In the early stages of infection, only severely inflamed endocervical cells are present. Later, the typical multinucleated giant epithelial cells with red intranuclear inclusions in their molded nuclei are frequently seen.[13,22] HPV is believed to be a promoter of the development of CIN and carcinoma cervix. The initial cytopathic changes in HPV infections are in the form of mild nuclear atypia and koilocytosis. Koilocyte is the most characteristic feature of HPV infection which is a squamous cell with three essential features, namely, abnormal nucleus, a large perinuclear halo or clear space, and a thickened uneven rim of dense cytoplasm.[13]

The precancerous changes are graded histologically as CIN Grades I, II, and III. Each of these grades is reflected by the type of abnormality seen on the cervical smear – mild, moderate, or severe dysplasia.[13] About 90% of the dysplastic lesions fall into the mild and moderate grades of CIN and the rest 10% fall into severe grades of CIN (carcinoma *in situ*).[16] CIN-I lesions are associated with low- and high-risk HPVs, whereas high grades of CIN are more strongly linked to high-risk HPV types.[16,23]

A pregnant woman should have a repeat smear taking after the birth of her baby. Those with high grades of dysplasia should have early surgical intervention after delivery and with few dysplastic changes on the background of chronic inflammation should have uterine cervical cytology with colposcopy-directed cervical punch biopsy at regular interval as a part of regular follow-up.[24,25]

Aims and Objectives

The objectives of the study were as follows:

- To determine the pattern of cervical smears (inflammatory vs. neoplastic) among the pregnant women attending the STD clinic at the institute of STDs.
- To study the characteristics of smears in relation to specific STIs among them.

MATERIALS AND METHODS

It is a cross-sectional study to find out the prevalence of pattern of cervical smears among pregnant women with or without STDs attending the STD clinic at Institute of Venereology, Madras Medical College and Research Institute, Chennai, during the study period of May 1998–July 1999. A total of 260 consecutive pregnant women at various trimesters who attended the STD clinic at the Institute of Venereology, Madras Medical College and Research Institute were included in the study. Majority of the unmarried pregnant women were referred to be screened for STIs including HIV infection, before medical termination of pregnancy. Many attended on their own due to symptoms. A well-structured, pre-tested pro forma was prepared and used for the study. It consists of various information including their age, socioeconomic status marital status, sexual history, and obstetric history apart from detailed clinic history. Pregnant women were screened for STDs including cervical smears and a provisional diagnosis was made. All other investigations were done accordingly to confirm the diagnosis.

RESULTS

In this study, 260 pregnant women were examined and the following results were obtained.

Age Distribution of Pregnant Women in the Study Group

Age group in years	Total no.	Percentage
15–19	48	18.5
20–24	144	55.4
25–29	50	19.2
30–34	16	6.2
35–39	2	0.8

Majority of pregnant women were in the group of 20–24 (55.4).

Socioeconomic Status of Pregnant Women

Monthly income (in rupees)	Total no.	Percentage
Up to 500	116	44.6
500–1000	74	28.5
1000–1500	32	12.3
1500–2000	18	6.9
Above 2000	20	7.7

Majority of pregnant belonged to lower socioeconomic strata.

Marital Status of the Pregnant Women in the Study Group

Marital status	Total no.	Percentage
Married	200	76.9
Single (unmarried)	60	23.1

Majority of the pregnant women were $n = 200$, 76.9%. In women, who were single ($n = 60$, 23.1%), majority were deserted by their known partner after knowing about the present pregnancy ($n = 50$, 83.3%), others forming the group were kept mistresses ($n = 6$, 10%), and commercial sex workers ($n = 4$, 6.6%).

Presenting Complaints of Pregnant Women

Complaints	Total no.	Percentage
Checkup	124	47.7
Genital discharge	72	27.7
Genital sore	34	13.1
Itching in genitalia	30	11.5
Growth in genitalia	20	7.7
Burning micturition	12	4.6
Lower abdominal pain	12	4.6
Fever	6	2.3
Frequency of micturition	4	1.5
Swelling in genitalia	4	1.5
Loss of weight	4	1.5
Cough	4	1.5
Loose stools	4	1.5
Loss of appetite	2	0.8
Dysuria	2	0.8

Majority of the pregnant women had visited the STD clinic for checkup ($n = 62$, 47.7%). Genital discharge and genital sore were the most common presenting complaints in 72 (27.7%) and 34 (13.1%) patients, respectively followed by itching in genitalia in 30 (11.5%) and growth in genitalia in 20 (7.7%) patients. Some pregnant women also had other symptoms such as burning micturition ($n = 12$,4.6%), lower abdominal pain ($n = 12$, 4.6%), fever ($n = 6$, 2.3%), cough ($n = 4$, 1.5%), low stools ($n = 4$, 1.5%), loss of weight ($n = 4$, 1.5%), frequency of micturition ($n = 4$, 1.5%), swelling of genitalia ($n = 4$, 1.5%), loss of appetite ($n = 2$, 0.8%), and dysuria ($n = 2$, 0.8%).

Premarital/extramarital Contacts among Married Pregnant Women ($n = 200$)

When married pregnant women were interviewed for this study, around 4% gave a history of (H/O) extramarital contact ($n = 8$) and 2% gave H/O premarital contact ($n = 4$). One woman (0.5%) admitted having both pre- and extra-marital contacts.

Number of Partner(S) among Unmarried/Single Pregnant Women ($n = 60$)

Group	Total no.	No. of partners	Percentage
Deserted	50	Single	83.3
Kept mistress	6	Single	10.0
Commercial sex workers	4	Multiple	6.7

Among unmarried pregnant, 25 women who were otherwise single gave H/O contact with a known

partner (83.3%). Four women (6.7%) who were commercial sex workers gave H/O contacts with multiple males. Six women (10%) who were kept mistress for a known partner denied H/O exposure outside.

Past History of STDs

Past history of STDs	Total no.	Percentage
Present	22	8.5
Absent	238	91.5

STDs: Sexually transmitted diseases

Distribution of Previous STDs (*n* = 22)

Disease	Total no.	Percentage
Genital ulcer	14	63.6
Genital discharge	6	27.3
Genital warts	2	9.1

STDs: Sexually transmitted diseases

BOH among the Pregnant Women (*n* = 68)

BOH	Total no.	Percentage
Spontaneous abortion	40	58.8
Still birth	16	23.5
Neonatal death	6	8.8
Spontaneous abortion and still births	6	8.8

Clinical Signs in the Study Group of Pregnant Women (*n* = 260)

Clinical sign	Total no.	Percentage
Cervical erosion	152	29.2
Soddening of vulva	100	19.2
Genital ulcer	72	13.8
Genital wart	40	7.7
Bilateral inguinal lymphadenopathy	28	5.4
Condylomata lata	16	3.1
Skin rash	12	2.3
Excoriations of vulva	8	1.5
Intertrigo groin	8	1.5
Molluscum contagiosum	4	0.8
Scabies	4	0.8
Tinea cruris	4	0.8

Cervical erosion was the most common clinical sign seen followed by soddening of the vulva.

Nature of the Genital Discharge among the Study Group (*n* = 260)

Nature of the discharge	Total no.	Percentage
Mucopurulent	104	40
Mucoid	96	36.9
Curdy white	52	20
Frothy	6	2.3
Serosanguinous	2	0.8

Majority of the pregnant women had mucopurulent discharge.

Results of Investigations in the Study Group (*n* = 260)

Endocervical and urethral smear gonococcus (Gram stain), and endocervical culture for gonococcus were negative in all pregnant women.

Investigations	Positive results (total no.)	Percentage
Culture of *Candida* spp. in SDA medium	124	47.7
Gram stain for candida	78	30
KOH mount for candida	56	21.5
Wet film for *Trichomonas vaginalis*	28	10.8
Gram stain for clue cells	6	2.3
Urine culture (routine)	6	2.3

VDRL Reactivity/TPHA

Serological test	Patients with reactive VDRL/ TPHA	Percentage
VDRL	66	25.4

Prevalence of HBs Ag

Serological test	Patients positive for HBs Ag	Percentage
HBs Ag	46	17.7

Prevalence of HIV Infection

Serological test	Total no. of patients positive for HIV antibodies	Percentage
HIV	16	6.2

Distribution of Infections among Pregnant Women

Infections	Total no.	Percentage
Vulvovaginal candidiasis	124	47.7
Syphilis	66	25.4
Hepatitis-B	46	17.7
Trichomoniasis	28	10.8
Genital herpes	26	10
Chlamydia cervicitis	21	8.1
Genital warts	20	7.7
HIV	16	6.2
Non-gonococcal urethritis	12	4.6
Bacterial vaginosis	6	2.3
Non-specific genital ulcer	4	1.5
Chancroid	2	0.8
Molluscum contagiosum	2	0.8
Scabies	2	0.8

Out of 66 patients with syphilis, 2 had primary syphilis (3%), 10 had secondary syphilis (15.2%), and 54 had yearly latent syphilis (81.8%).

Total Number of Cervical Smears Examined = 260

Cytological findings	Total no.	Percentage
Normal smear	60	23.1
Neutrophilic (acute) inflammatory smear with *Candida*	50	19.2
Neutrophilic inflammatory smear with microorganisms in the background	46	17.7
Neutrophilic inflammatory smear with Grade 1 dysplasia	22	8.5
Neutrophilic inflammatory smear with degenerative epithelial cell changes	16	6.2
Neutrophilic inflammatory smear with Grade 1 dysplasia with *Candida*	14	5.2
Neutrophilic inflammatory smear with degenerative epithelial cells+*Candida*	6	2.3
Neutrophilic inflammatory smear with intranuclear inclusion bodies and giant epithelial cell with polynucleation	6	2.3
Neutrophilic inflammatory smear with degenerative epithelial cell changes with Grade 1 dysplasia	4	1.5
Neutrophilic inflammatory smear+Grade 1 dysplasia+koilocytosis	4	1.5
Neutrophilic inflammatory smear+Grade 1 dysplasia+*Candida*+koilocytes	4	1.5
Neutrophilic inflammatory smear with trichomonads	4	1.5
Neutrophilic inflammatory smear with trichomonads and *Candida*	4	1.5
Mixed inflammatory smear with Grade 1 dysplasia	4	1.5
Clue cell alone	4	1.5
Clue cells with Grade I dysplasia	2	0.8
Chronic inflammatory smear with microorganisms in the background	2	0.8
Chronic inflammatory smear with Grade I dysplasia with koilocytes	2	0.8
Chronic inflammatory smear with Grade II dysplasia	2	0.8
Mixed inflammatory smear with microorganisms in the background	2	0.8
Neutrophilic inflammatory smear with herpetic nuclear inclusion bodies and *Candida*	2	0.8

DISCUSSION

Majority of the pregnant women had visited the STD outpatient department for checkup. Most of them were referred cases. A good number of pregnant women presented with various genitourinary symptom on their own along with their partners. This increase in number of self-referral by these women could be as a result of campaigns conducted for generating awareness about STDs including HIV infection.

Nearly half of the pregnant women reported only for checkup. However on clinical examination and completion of investigations, it was found that 230 (88.5%) pregnant women were found to be suffering from one or other infections. This shows that majority of the pregnant women were asymptomatic. Genital infections often remain unnoticed during pregnancy as their signs and symptoms may be seen as part of the normal discomfort of pregnancy. The asymptomatic nature of infections in females and their frequent consultation in gynecological clinics instead of STD clinics may have been responsible for a low prevalence of STD reported in women.[26] Genital discharge, genital sore, and itching in genitalia were the most common symptoms noticed among the study group. Genital ulcer was the most common among the previous STDS noted. We also noted the increased rate of recurrence of genital herpes during pregnancy, similar to the observers.[19,27] In the study group, cervical erosion, soddening of vulva, genital ulcer, and genital warts were the important clinical signs noted. Erosion of cervix was seen in nearly one-third of pregnant women. Erosion or cervical ectopy enhances the entry of a variety of pathogenic agents.

In this study, mucopurulent vaginal discharge was commonly seen. Vaginal discharge apart from vaginal infection may also be caused by mucopurulent cervicitis, so it is essential to examine cervix of all patients with vulvovaginal complaints using speculum. Patients with *T. vaginalis* vaginitis (n = 14) had predominantly mucopurulent discharge and the typical frothy discharge of trichomoniasis was seen only in three. The usual description of discharge in vulvovaginal candidiasis is curdy white and adherent. Only 20% of the pregnant women had the above said clinical sign. Majority of these patients had no symptoms while the rest had itching in genitalia. The clinical presentation is similar in both HIV-infected and seronegative pregnant women although severity of symptoms was noted in the former.

Three patients (2.3%) had BV and their genital discharge was mucoid in nature. Prevalence of BV in our study was low compared to an American study which quotes prevalence of 15–20%. Serosanguineous discharge was noted in a pregnant woman with extensive genital herpes. Culture from the endocervical canal for gonococcus was negative in all the study subjects. Non-gonococcal urethritis was seen in twelve pregnant women (*Escherichia coli* was the most common isolate).

The prevalence of genital herpes in the study population was 10%. Most of these cases were due to recurrences of HIV infection, especially in the third trimester. Low prevalence of genital herpes is comparable with other studies.[28] The prevalence of genital warts in the group of pregnant women studied was 7.7%. This was almost comparable to the existing data available.[29] Extensive huge, anogenital warts were noted in the HIV-positive pregnant women. Only one in the study had previous the history of condyloma acuminata.

Prevalence of trichomoniasis in the study population was 10.8%. This corroborates with the reported prevalence data.[2,7,30] About 50% of the pregnant women had genital candidiasis.

Among the cervical smears of pregnant women studied (n = 260), majority of them were inflammatory in nature (n = 194, 76.4%). Dysplastic changes could be noticed in 58 (22.3%) and degenerative epithelial cell changes could be found in 26 (10%) smears. Features of acute inflammation were seen in 182 (93.8%) smears [Figure 1] and chronic inflammation in 6 (3.1%). Both features were found in 6 smears (3.1%). Among the inflammatory smears (n = 194), *Candida* spores/pseudohyphae were seen in 80(41.2%), trichomonads in 8(4.1%), herpetic intranuclear inclusion bodies and multinucleated giant epithelial cells in 8 (4.1%), and koilocytes in 10 (5.2%) smears [Figure 2]. Clue cells were seen in 6 cervical smears (2.3%). Out of these, one smear showed Grade 1 dysplasia (33.3%). Those showing dysplastic features, Grade 1 dysplasia [Figure 3] was noted in 54 (27.8%) and Grade 2 dysplasia was found in 2 smears (1%). Acute inflammatory and chronic inflammatory pictures were noted in this study. Grades 1 and 2 dysplastic changes were noticed in chronic HSV/HPV infections [Figure 4]. Severe dysplastic changes in few areas of the smear were also noticed. Normal smear was also observed in significant numbers in the study group. Hence, we suggest that colposcopic-directed cervical biopsy can be used to further investigate and follow-up in the postpartum period due to high persistent rate of CIN.

Cytologic Findings in Patients with HPV Infection

The cervical smears (H and E stain) showed koilocytosis (n = 10), inflammatory changes (acute in 12, chronic in 4, and both in 4), and degenerative epithelial cell changes (n = 4). Dysplastic changes were noted in 12 smears (Grade 1 in 10 and Grade 2 in two).

Cytologic Findings in Patients with HSV Infection

The cervical smears (H and E stain) showed multinucleated giant epithelial cells and intranuclear bodies (n = 8), inflammatory changes mainly acute in majority. Degenerative epithelial changes were noted in four and dysplastic changes in 10 patients.

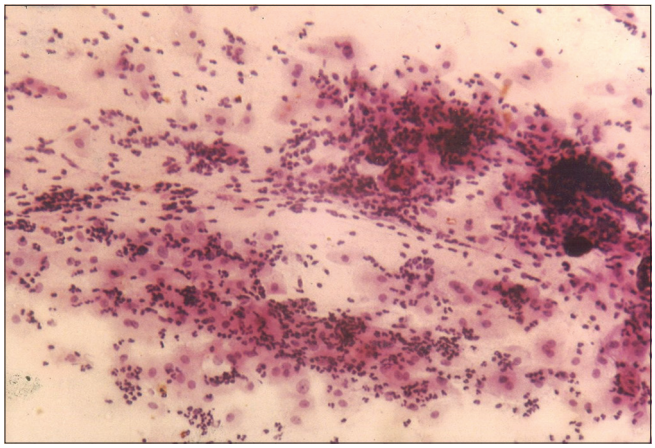

Figure 1: Cervical smear (H and E stain) showing sheets of polymorphs indicative of acute inflammation in a woman with candidiasis and genital herpes

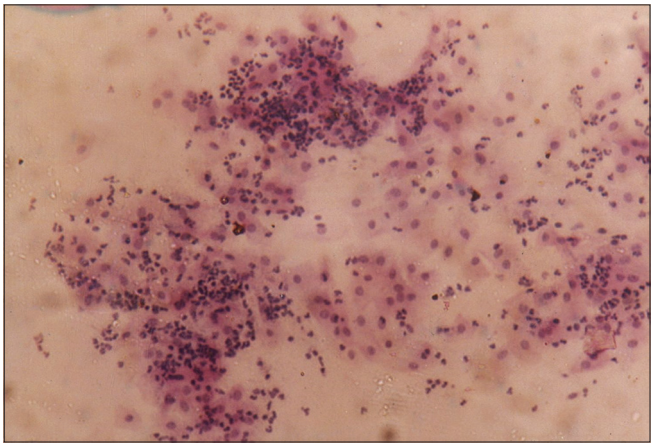

Figure 3: Cervical smear (H and E stain) showing Grade 1 dysplasia in low magnification in a pregnant woman with genital warts

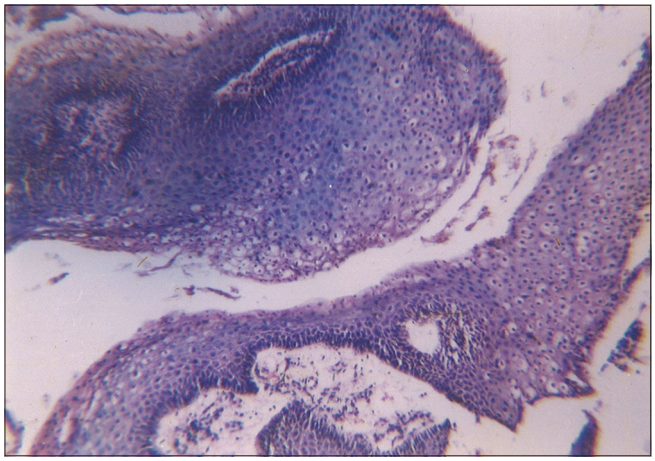

Figure 2: Koilocytosis in HPE of genital warts

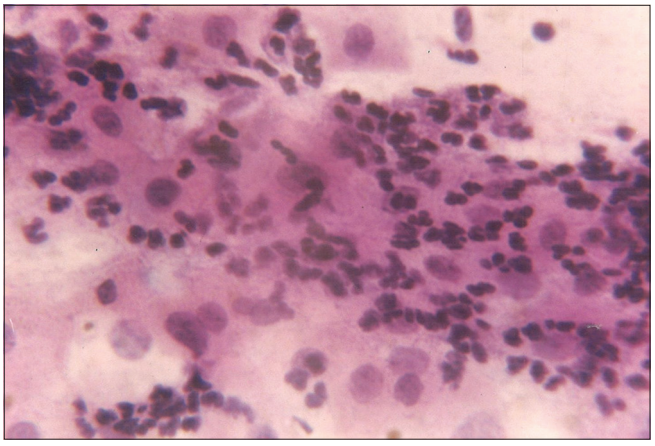

Figure 4: Cervical smear (H and E stain) with dysplastic changes

Cytologic Findings in Patients with Bacterial Vaginosis

The cervical smears (Hand E stain) showed clue cells and did not show any inflammatory changes. Two patients had features of Grade 1 dysplasia.

Cytologic Findings in Patients with Trichomoniasis

The cervical smears (Hand E stain) showed mainly inflammatory cells consisting of polymorphs. Dysplastic changes were noted in four study subjects.

Cytologic Findings in Patients with Candidiasis

The cervical smears (Hand E stain) showed mainly inflammatory cells consisting of polymorphs. Degenerative changes were noted in three study patients.

Cytologic Findings in Patients with Syphilis

Majority of the smears (Hand E stain) showed acute inflammatory changes. Two patients who had coexisting HIV infection had a chronic inflammatory picture. Six patients showed features of mild dysplasia.

Cytologic Findings in Patients with Chancroid

Majority of the smears (Hand E stain) showed acute inflammatory changes.

CONCLUSION

The large numbers of pregnant women with an STD provide an important opportunity to reduce the reservoir of infection in the broader community. Hence, systematic screening of STIs which may include cervical smears to detect early neoplastic changes in pregnancies combined with adequate treatment and follow-up (colposcopic-directed cervical biopsy later on) will reduce the risk of adverse consequences. Appropriate antenatal, intrapartum, and postnatal care should be provided for those with chronic inflammatory and early carcinomatous changes in cervical study. Chronic viral infections such as warts and herpes should be actively managed with frequent clinical follow-up for warning signals as they often predispose to cervical cancer. Chronic cervicitis cases should have cytology at constant intervals to detect early atypia. Colposcopically directed biopsy which is one of the safe and reliable methods can be employed to further evaluate those with abnormal cervical cytology.

To conclude, there is an urgent need to mount effective, rational, and feasible intervention programs to combat the raising prevalence of carcinoma cervix in Indian women with early screening programs in antenatal mothers (first regular visits to any medical care facilities) so as to enroll them for regular care and follow-up, making optimum use of the existing health and social welfare services.

REFERENCES

1. Dallabetta GA, Laga M, Lamptey P. Control of Sexually Transmitted Diseases. AIDSCAP/Family Health International USA; 1996. p. 169-86.
2. Passey M, Mgone CS, Lupiwa S. Screening for STDs in rural women in Papua New Guinea. Bull World Health Organ 1998;76:401-11.
3. Bourgeois A, Henzel D, Malonga-Mouelet G. Clinical algorithms for the screening of pregnant women for STDs in Libreville, Gabon-which alternatives? Sex Transmit Infect 1998;74:35-9.
4. Hall AJ, Peckham CS. Infections in childhood and pregnancy as a cause of adult disease methods and examples. Br Med Bull 1997;33:10-23.
5. Heller J. Violence and reproductive health. World Health 1998;51:13-4.
6. Menon MK, Devi PK, Rao KB. Postgraduates Obstetrics and Gynaecology. 4th ed. Andhra Pradesh: Orient Longman Publishers; 1991. p. 367.
7. Holmes KK, Mardh PA, Sparling PF. Sexually Transmitted Diseases. 3rd ed. New York: McGraw Hill International; 1999. p. 1089-132.
8. Cheery SH, Merkat IR. Infections in Pregnancy Complications of Pregnancy Medical, Surgical, Gynaecological, Psychosocial And Perinatal. 4th ed. Williams/Wilkins; 1991. p. 302-83.
9. Jaiswal AK, Banerjee S. Changing trends in STDs in North Eastern India. Indian J Dermatol Venereol Leprosy 2002;68:65-6.
10. Brabin L, Gogate A, Karande A, Khanna R, Dollimore N, de Koning K. Reproductive tract infections, gynaecological morbidity and HIV seroprevalence among women in Mumbai, India. Bull World Health Organ 1998;76:277-87.
11. Taha E Taha, Guna A Dallabetta. Trends of HIV-1 and sexually transmitted diseases among pregnant and postpartum women in urban Malawi. AIDS 1998;12:197-203.
12. Atkinson BF, Silverman JF. Cytological techniques, artefacts and special procedures. In: Atlas of Difficult Diagnosis in Cytopathology. 1st ed. Philadelphia, PA: WB Saunders Company; 1998.
13. Mackee GT. Cytopathology. 1st ed. Maryland Heights, Missouri: Mosby Wolfe, Times Mirror International Publishers Limited; 1997. p. 13-63.
14. Stack PS. Pap Smears. Postgrad Med 1997;101:207-14.
15. Wilson JD, Parsons W. Cervical cytology smears in sexually transmitted infection clinics in the United Kingdom. Sex Transm Infect 2001;77:107-10.
16. Silverbag SG, Frable WJ. "The Uterine Cervix" Principles and Practice of Surgical Pathology. 2nd ed., Vol. 3. London, United Kingdom: Churchill Livingston Publishers; 2013. p. 2343-409.
17. Efwards SK, Sonnex C. Influence of genital infection on cervical cytology. Sex Transmitt Infect 1998;74:271-3.
18. Gorisek T. Chlamydia trachomatis infection in women with and without cervical intraepithelial neoplasi. Int J STD and AIDS 1999;10:331-3.
19. Martens KA. Sexually transmitted and genital tract infections during pregnancy. Emerg Med Clin North Am 1994;12:91-113.
20. Herzberg AJ, Silverman JF. Detection of *Trichomonas vaginalis* in endocervicaland ectocervical smears. Diagn Cytopathol 1996;14:273-6.
21. Woodrow N, Lamont RF. Bacterial vaginosis its importance in obstetrics. Hosp Med 1998;59:447-50.
22. Rao KB, Ratnam SS, Arulkumaran S. Obstetrics and Gynaecology For postgraduates. 1st ed., Vol. 1. Hyderabad: Orient Longman Publishers; 1996.
23. Silverbag SG, Frable WJ. "The Uterine Cervix" Principles and Practice of Surgical Pathology. 2nd ed., Vol. 3. London, United Kingdom: Churchill Livingston Publishers; 2013. p. 2343-409.
24. Morimura Y, Fujimori K, Soeda S, Hashimoto T, Takano Y, Yamada H, *et al*. Cervical cytology during pregnancy--comparison with non-pregnant women and management of pregnant women with abnormal cytology. Fukushima J Med Sci 2002;48:27-37.
25. Kashimura M, Matsuura Y, Shinohara M, Baba S, Obara K, Fujiwara H, *et al*. Comparative study of cytology and punch biopsy in cervical intraepithelial neoplasia during pregnancy. A preliminary report. Acta Cytol 1991;35:100-4.
26. Khandpur S, Agrawal S. Clinico-epidemiological profile and HIV seropositivity of STD patients. Indian J Sex Transmit Dis 2001;22:62-5.
27. Lamont RF, Hudson EA. A comparison of the use of Papanicolaou stained cervical cytological smears with gram stained vaginal smears for the diagnosis of bacterial vaginosis in early pregnancy. Int J STD AIDS 1999;10:93-7.
28. Bhargava RK. Viral STDs. In: IADVL Text Book and Atlas of Dermatology.

2nd ed., Vol. 2, Ch. 58. New Delhi: Jaypee Publishers; 2001. p. 1476-90.
29. Obstetrics and Gynaecology Communications. HPV Infection in Cervical Carc Inogeneisis. Vol. 2. 1999. p. 15-22.
30. Ronald S, Gibbs MD, Richard L, Sweet MD. Maternal and fetal infectious disorders. In: Maternal and Fetal Medicine. 4th ed. Philadelphia, Pennsylvania: WB Saunders; 2002.

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Original Article

A Cross-sectional Questionnaire-based Survey of Difficulties Faced Post-COVID-19 among Patients Treated at a COVID Jumbo Facility in Mumbai Region

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Abstract

Background and Objectives: Persistent symptoms after COVID-19 infections are emerging as a new challenge to healthcare workers of which there is sparse documentation in the literature. The objective of this study is to evaluate the persistent symptoms and the attitude of the individuals who have recovered from COVID-19 infection.

Methodology: 474 fully recovered individuals who were hospitalized with mild or moderate COVID infection were included in the study. A questionnaire-based analysis to determine the persistent symptoms and their attitude was conducted during the follow-up evaluation at a jumbo COVID facility of Mumbai metropolitan region. The participant response was electronically recorded and qualitatively analyzed.

Results and Discussion: Among the study participants, it was found that around 41% presented with at least one persistent symptom during the follow-up. The predominant symptoms that persisted are fatigue (41.6%), cough (16.9%), burning in retrosternal area (11%), and body ache (9.9%). Burning sensation in the retrosternal area is a new symptom which was not observed in the participants at the time of hospitalization.

Conclusion: Based on the study finding of the persistent symptoms in post-COVID recovered patients, a multi-disciplinary clinical approach is needed to manage the physical symptoms as well as the psychological aspect of the individuals.

Key words: COVID-19, Long COVID, Persistent symptoms

INTRODUCTION

Sever acute respiratory syndrome coronavirus-2 (SARS-COV-2) is a novel coronavirus infection mainly affecting the respiratory system, which was considered as the primary cause for the outbreak of COVID-19 over the world.[1] Since its initial isolation in December 2019 from Wuhan, China the infection has spread to majority of the countries and infected millions worldwide. The term "coronavirus" is derived from the Latin word CORONA meaning "crown" as its structure resembles a crown with multiple spikes under an electron microscope.[2] SARS-COV-2 is an enveloped positive sense single-stranded RNA virus with multiple spikes on the surface having a genome size of approximately 26–32 kilobases and belonging to the genera beta coronavirus.[3]

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As per the latest statistics of the World Health Organization (WHO), the number of infected people in the world is close to 186 million with over 30 million infected in India.[4] The death count of individuals affected by COVID-19 has crossed 4 million.[4] The mortality rate of SARS-COV-2 is lesser compared to the previous infections of SARS and Middle East respiratory syndrome but a high human to human transmission rate.[5] The incubation period for COVID-19 infection is typically 1–14 days which may extend up to 24 days.[6] Most human cases of COVID-19 are mild with 5% of the infected patients developing

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severe symptoms that requires mechanical ventilation and intensive care hospitalization. The most common symptoms are fever, dry cough, shortness of breath, dysosmia, and dysgeusia.[7] In addition, the gastrointestinal system has been found to be affected in many patients.[8] The clinical symptoms of COVID-19 are broadly classified into three classes. The most common are fever, dry cough, and tiredness. Less common symptoms are body aches, sore throat, diarrhea, loss of taste or smell and severe symptoms are difficulty in breathing or shortness of breath, chest pain or pressure, and loss of speech or movement.[9]

While most of the patients recover from the COVID-19 infection, there are few who continue to experience post-COVID conditions. Post-COVID conditions are a wide range of new, returning or on-going health problems people can experience four or more weeks after being first infected with COVID-19. These post-COVID conditions may also be known as long COVID, long-haul COVID, post-acute COVID (symptoms beyond 3–4 weeks), long-term effects of COVID or chronic COVID (symptoms beyond 12 weeks).[10] The symptoms of long COVID can be encountered not only in patients with severe COVID-19 infection, but also in patients who showed mild symptoms and did not required hospitalization. These patients may experience an array of persistent symptoms that may include cough, breathlessness, fever, sore throat, chest pain, palpitations, and multi-organ involvement among others.[11]

At present, there is minimal data concerning the occurrence of post-COVID symptoms and hence it is difficult to understand the long-term health consequences of COVID-19 infection. Obtaining and reporting data related to the occurrence of post-COVID symptoms from hospitalized as well as non-hospitalized patients is the need of the hour that may help us to understand the full spectrum of COVID-19 infection and also aid in framing appropriate treatment and preventive strategies. Thus, the aim of the present study is to evaluate the persistent post-COVID symptoms in fully recovered hospitalized patients; the mental status of patients post-COVID recovery; and the probable application of some social policies for the betterment of their post-COVID lifestyle.

METHODOLOGY

A cross-sectional questionnaire-based survey was conducted at a COVID-19 care facility in Mumbai district to identify the post-COVID complications of COVID-19 infected individuals. The center was established at the time of emergence of first wave of COVID-19 during May 2020 with the objective of treating COVID-19 infected patients of all severity. In addition, the center also had a dedicated service available for follow-up evaluation of treated patients. For the study, a structured questionnaire was administered on the eligible participants and their responses was recorded in electronic format. Questions were focused on collecting demographic data, symptoms with which the patient presented at the time of diagnosis of COVID-19, the persistent symptoms and others during the follow-up examination of recovered patients. Inclusion criteria included those patients who were initially diagnosed with COVID-19 infection based on the symptoms as per the WHO criteria and positive reverse transcription polymerase chain reaction test for SARS-COV-2 infection and were categorized as mild and/or moderate infection that required hospitalization. On admission, the clinical symptoms were recorded along with the lab investigations, as necessary. The patients were discharged on completion of their quarantine period as well as other criteria as per the WHO guidelines for discharge of patients on completion of treatment. After discharge, these patients were then recalled for a follow-up evaluation after 8 weeks. At the time of follow-up evaluation, the study participants were selected by random sampling irrespective of age, gender, and socio-economic status. A total of 474 eligible participants were enrolled in the study. The study participants were provided with all information regarding the study and informed consent was obtained from them before providing the questionnaire. The study participants were then asked to respond to the questions as provided in the questionnaire that were stored anonymously in an electronic mode. Exclusion criteria included those patients who were admitted with severe symptoms during first visit that required intensive care, as well as the participants who provided incomplete questionnaire. The obtained data were then tabulated and qualitatively analysed for the responses obtained from the participants.

RESULTS

The study was performed during the time frame of 4 months (December 2020 to March 2021). A total of 474 participants responded to the questionnaire to evaluate the symptoms and the attitude of individuals post-recovery from COVID-19 infection. The median age of the participants was 51 years (Range: Min- 7 years; Max- 80 years) and included 66.9% males (n = 317) and 33.1% (n = 157) were females. Majority of the participants required hospitalization for treatment and the common symptoms encountered at the time of admission included fever (n = 457), sore throat (n = 372), cough (n = 344), breathlessness (n = 345), and fatigue (n = 130) [Table 1]. Less common symptoms included nausea, vomiting, loss of taste, and abdominal pain among others.

Data analysis of the respondents at the time of follow-up revealed that around 41% of the participants had one or more symptoms that persisted even after 8 weeks of discharge. The predominant symptoms that persisted are fatigue (41.6%), cough (16.9%), burning in retrosternal area (11%), and body ache (9.9%) [Table 2]. Burning sensation in the retrosternal area is a new symptom which was not observed in the participants at the time of hospitalization.

The data obtained from the study suggested the long-term persistence of COVID-19 symptoms. In addition, the discomfort associated with the persistent symptoms was hindering the quality of life and work output to some extent although it did not have any severe consequences.

Table 1: Demographic and clinical characteristics of the study participants (*n*=474)

Characteristics	Value
Age	
Median	51
Range	7 to 80
Sex (%)	
Male	66.9 (*n*=317)
Female	33.1 (*n*=157)
Symptoms at the time of diagnosis (%)	
Cough	344 (72.6)
Sore throat	372 (78.5)
Fever	457 (96.4)
Dyspnea/Breathlessness	345 (72.8)
Fatigue	130 (27.45)
Nausea	37 (7.8)
Vomiting	32 (6.8)
Abdominal pain	25 (5.3)
Loss of taste	17 (3.6)
Headache	17 (3.6)
Chest Pain	0
Lack of appetite	0
Loose motions	10 (2.1)
Joint Pain	4 (0.8)
Bodyache	13 (2.7)
Any other (cold)	3 (0.6)
Hospitalization required (%)	
Yes	98.7
No	1.3
Home quarantine (%)	
Yes	1.3
No	98.7
Persistent symptoms at the time of follow up (%)	
Cough	80 (16.9)
Sore throat	19 (4)
Fever	10 (2.1)
Dyspnea/Breathlessness	12 (2.5)
Fatigue	197 (41.6)
Nausea	2 (0.4)
Vomiting	0
Abdominal pain	3 (0.6)
Loss of taste	2 (0.4)
Headache	5 (1.1)
Chest Pain	3 (0.6)
Lack of appetite	2 (0.4)
Loose motions	4 (0.8)
Burning in retrosternal area	53 (11.2)
Joint Pain	17 (3.6)
Bodyache	47 (9.9)
Any other	Remaining
Pain/discomfort preventing from daily work (%)	
Not at all	18.8
A little	81.3
Moderate	------
Very much	------
Extreme amount	-----
Quality of life post-COVID-19 recovery (%)	
Very poor	------
Poor	------
Good	52.1
Very good	------
Neither poor nor good	46.8

DISCUSSION

This qualitative cross-sectional study was conducted among participants to evaluate the persistent symptoms and the general attitude of 474 study participants recovered from COVID-19 infection which revealed certain important findings. Most of the participants included in the study required hospitalization but not intensive care and were diagnosed with mild to moderate infection. The predominant symptoms at the time of hospitalization included fever, sore throat, cough, breathlessness, and fatigue. The patients were discharged a per the WHO criteria for patient discharge and were advised follow-up evaluation. During the follow-up examination and questionnaire survey, it was observed that around 41% of the study participants had at least one persistent symptom. The primary persistent symptoms were fatigue, cough, body ache, and burning in retrosternal area. This finding was in accordance with the literature data available till date. It is also noteworthy that symptoms such as burning

Table 2: COVID-19 related symptoms at hospitalization and follow-up period

Symptoms	Baseline, Hospitalization (%)	Symptoms reported at the follow-up period after recovery (%)
Cough	344 (72.6)	80 (16.9)
Sore throat	372 (78.5)	19 (4)
Fever	457 (96.4)	10 (2.1)
Dyspnoea/Breathlessness	345 (72.8)	12 (2.5)
Fatigue	130 (27.45)	197 (41.6)
Nausea	37 (7.8)	2 (0.4)
Vomiting	32 (6.8)	0
Abdominal pain	25 (5.3)	3 (0.6)
Loss of taste	17 (3.6)	2 (0.4)
Headache	17 (3.6)	5 (1.1)
Chest Pain	0	3 (0.6)
Lack of appetite	0	2 (0.4)
Loose motions	10 (2.1)	4 (0.8)
Burning in retrosternal area	0	53 (11.2)
Joint Pain	4 (0.8)	17 (3.6)
Body ache	13 (2.7)	47 (9.9)
Any other (cold)	3 (0.6)	Remaining

sensation in retro-sternal area were present at follow-up evaluation but were not observed in any of the participants at the time of hospitalization during the initial diagnosis.

A study performed by Leth *et al.* (2021) has found persistent symptoms such as fatigue, dyspnea, cough, chemosensory dysfunction, and headache in 96% of the participants even after 12-week follow-up.[12] Another study by Carfì *et al.* (2020) found that only 13% of the study population were free of any symptoms after 60 days follow-up and the symptoms were similar to the findings of our study.[11] Based on the presence of the persistent symptoms; in our study, it was noted that these symptoms originated from multiple organ systems. The involvement of multiple organ system could possibly reflect the wide distribution of the angiotensin-converting enzyme 2 in different tissues such as the epithelium of the intestine, kidney, and blood vessels among others.[13] Further it is possible that the inflammatory response which is accentuated secondary to damage of the epithelial-endothelial barrier due to acceleration of the viral replication could contribute to the wide range of persistent symptoms in patients recovering from COVID-19.[12]

Based on the findings of the present study as well as the literature data, it is evident that symptoms related to COVID-19 are known to persist even after the patient recovery from the infection which may have huge health and socio-economic consequences. However, there is lack of adequate knowledge regarding the pathogenesis or the organs involved in persistent COVID-19 symptoms. To provide adequate care for these individuals requires a dedicated, multi-disciplinary clinical approach to reduce the long-term consequences of the illness and also to improve the post recovery quality of life. The need of the hour is to perform more such studies involving large study population which should involve both hospitalized and non-hospitalized individuals, the data obtained from which can be used to frame appropriate guidelines for managing post-COVID symptoms. Further, specialized centers should be set up for investigation and management by adequately trained health care workers.[14]

In addition to the assessment of the persistent symptoms, this study also evaluated the attitude of the recovered COVID-19 individuals. It was observed that while many of the recovered patients graded that the quality of life was good, around 47% of the study participants were not sure about the same. On further insistence regarding the question on quality of life, many of them mentioned that they were simply happy to be alive. During the personal interaction with the participants, majority of them showed anxiety related to the recovery and quality of life as well as fear of recurrence of infection. Furthermore, stress related to losing out on jobs or family was also evident in some of the participants.

Cumulatively, it has been observed that the individuals who have recovered from COVID-19 not only encounter persistent physical symptoms, but also show varying levels of psychological trauma. Instances of post-traumatic stress disorders, depression and anxiety have been reported to occur especially in patients with severe infection. However, not much studies have focused on the psychological aspect of COVID-19 recovered individuals.

CONCLUSION

The present study is one of the few documentations of a large sample size from a single center dedicated for COVID-19 management. From the study, it has been observed that the most common post-COVID symptom was fatigue (41.6%) which they encountered during routine work followed by persistent cough, burning in retrosternal area and body ache. Anxiety pertaining to quality of life and family was observed in many participants. Overall, there is an urgent need for longitudinal, multi-national and multi-site studies focusing on both the physical symptoms and mental status. Further, exclusive centers should be set up which caters to the management of persistent symptoms and also provide psychological counseling to those needful of the same. A holistic management approach that includes healthy diet, intake of nutritional supplements and having a right balance between the physical and mental activities could be helpful.

REFERENCES

1. Mohamadian M, Chiti H, Shoghli A, Biglari S, Parsamanesh N, Esmaeilzadeh A. COVID-19: Virology, biology and novel laboratory diagnosis. J Gene Med 2021;23:e3303.
2. Singh G, Priya H, Mishra D, Kumar H, Monga N, Kumari K. Oral manifestations and dental practice recommendations during COVID-19 pandemic. J Family Med Prim Care 2021;10:102-9.
3. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, *et al*. Structure of the sars-cov-2 spike receptor-binding domain bound to the ace2 receptor. Nature 2020;581:215-20.
4. Available from: http://www.covid19.who.int. [Last accessed on 2021 Sep 08].
5. Alsharif W, Qurashi A. Effectiveness of COVID-19 diagnosis and management tools: A review. Radiography 2021;27:682-7.
6. Lauer S, Grantz K, Bi Q, Jones F, Zheng Q, Meredith H, *et al*. The incubation period of Coronavirus disease (COVID-19) from publicily reported confirmed cases: Estimation and application. Ann Intern Med 2020;172:577-82.
7. Dos Santos JA, Normando AG, da Silva RL, Acevedo AC, de Luca Canto G, Sugaya N, Santos-Silva AR. Oral manifestations in patients with COVID-19: A livins systematic review. J Dent Res 2021;100:141-54.
8. Sulaiman T, Algharawi AA, Idrees M, Alzaidy RH, Faris K, Cullingford G, *et al*. The prevalence of gastrointestinal symptoms among patients with COVID-19 and the effect on the severity of the disease. JGH Open 2020;4:1162-6.
9. Kar S. COVID-19: A brief clinical overview. J Geriatr Care Res 2020;7:74-8.
10. Ladds E, Rushforth A, Wieringa S, Taylor S, Rayner C, Husain L, *et al*. Persistent symptoms after COVID-19: Qualitative study of 114 "long COVID" patients and draft quality principles for services. BMC Health Serv Res 2020;20:1144.

11. Carfì A, Bernabei R, Landi F. Persistent symptoms in patients after acute COVID-19. JAMA 2020;324:603-5.
12. Leth S, Gunst JD, Mathiasen V, Hansen K, Søgaard O, Østergaard L, *et al*. Persistent symptoms in patients recovering from COVID-19 in Denmark. Open Forum Infect Dis 2021;8:ofab042.
13. Cevik M, Kuppalli K, Kindrachuk J, Peiris M. Virology, transmission, and pathogenesis of SARS-CoV-2. BMJ 2020;371:m3862.
14. Gemelli against COVID-19 Post-Acute Care Study Group. Post-COVID-19 global health strategies: The need for an interdisciplinary approach. Aging Clin Exp Res 2020;32:1613-20.

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Original Article

A Quantitative Analytical Study of the Prevailing Prescriptions of Dipeptidyl Peptidase 4 Inhibitors, Sitagliptin and Gemigliptin, among Type II Diabetes Mellitus Patients of Tertiary Care Hospitals

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Abstract

Introduction: Diabetes mellitus type II is globally very common, yet neglected. Inhibition of dipeptidyl peptidase-4 by DPP-4 inhibitors enhances hormonal activity of incretins (GLP-1, GIP, and GRP), stimulates insulin release, and reduces glucagon secretion, thus producing anti-hyperglycemic activity in type II diabetes mellitus patients.

Objective: The objective is a quantitative analytical study of the prevailing prescriptions of dipeptidyl peptidase 4 inhibitors, sitagliptin and gemigliptin, among type II diabetes mellitus patients of tertiary care hospitals.

Materials and Methods: A total of 250 new early moderate grade type II diabetes mellitus patients were prescribed oral sitagliptin 25 mg once daily or gemigliptin 25 mg once daily for 3 months, in monotherapy, or in combination therapy, or in a mixed regimen of monotherapy and combination therapy, with another oral hypoglycemic drug. The safety and efficacy assessments, with blood sugar and HbA1c levels and urine routine examination, at subsequent intervals and follow-up, were recorded and statistically analyzed. The number of prescriptions for each drug was recorded, and the corresponding prescription rates were statistically analyzed in percentages.

Results: Sitagliptin was most commonly prescribed (200 prescriptions, 80%) followed by gemigliptin (50 prescriptions, 20%).

Conclusions: Prescription frequency of sitagliptin was followed by gemigliptin.

Key words: Dipeptidyl peptidase-4 inhibitors, Sitagliptin, Gemigliptin, Prescribing patterns

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INTRODUCTION

Diabetes mellitus type II is one of the universally prevalent common, yet often neglected, disease that the world has witnessed in the recent times. The incidence and prevalence of type 2 diabetes mellitus (T2DM) are increasing globally, with about one in 11 adults having

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diabetes mellitus and 90% of them having T2DM. According to the International Diabetes Federation, 425 million people worldwide have diabetes mellitus, accounting for two-thirds of adults aged 20–64 years, and the proportion of deaths due to diabetes mellitus before the age of 60 years ranges from 36 to 73%. The 10 countries with the highest prevalence of diabetes mellitus account for almost 60% of the global disease burden, with China (114 million people), India (73 million people), and the USA (30 million people) contributing to most of this. Therefore, the management of diabetes mellitus through effective treatment interventions is of the utmost importance in the field of clinical research.[1,2]

Inhibition of dipeptidyl peptidase-4 by dipeptidyl peptidase-4 inhibitors enhances the hormone activity of incretins, such as glucagon like peptide-1 and other bioactive peptides (glucose-dependent insulinotropic polypeptide and gastrin-releasing peptide), thus stimulating the release of insulin and reducing the secretion of glucagon, when given in monotherapy or in combination with metformin. This effect decreases the blood glucose levels as well as HbA1c levels in type II diabetes mellitus patients, without causing severe hypoglycemia.[3,4]

Objective

The objective is a quantitative analytical study of the prevailing prescriptions of dipeptidyl peptidase 4 inhibitors, sitagliptin and gemigliptin, among type II diabetes mellitus patients of tertiary care hospitals.

MATERIALS AND METHODS

Ethical Approval

At first, the Institutional Ethics Committee clearance and approval was taken. The study was conducted in accordance with the ethical principles of Declaration of Helsinki and Good Clinical Practices contained within the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH-E6) and in compliance with the regulatory requirements. An informed consent was obtained from each patient.

Inclusion Criteria

The inclusion criteria were as follows: (i) Patients of any gender, (ii) patients within 35 and 60 years, (iii) patients presenting with new type II diabetes mellitus, of early moderate grade, (iv) type II diabetes mellitus American Diabetes Association diagnosis criteria, (v) cooperative and conscious patients, (vi) patients willing to undergo all pre- and post-treatment investigations and willing to complete the entire course of treatment, (vii) patients who have given consent and are willing to go for a follow-up, (viii) patients not taking any previous antidiabetic drug, and (ix) patients not taking any concomitant medication.

Exclusion Criteria

The exclusion criteria were as follows: (i) Uncooperative or unconscious patients, (ii) patients below 35 and above 60 years, (iii) patients presenting with any grade other than early moderate grade of diabetes, (iv) patients with a history of hypersensitivity to any of the study drugs, (v) patients with high-risk diseases or comorbidities, (vi) cardiac, renal, or any other associated complications or comorbidities, (vii) any chronic disease intervening with the study data, (x) pregnant or lactating women, (xi) pediatric or geriatric patients, (xii) other associated medical illness or disorders, having impact on study results, and (xiii) female patients using hormonal contraceptives.

Study Design

A global, multicenter, retrospective, observational, and analytical study of the clinical prescriptions was performed.

Study Population

The study population consisted of 250 treated new type II diabetes mellitus patients, of early moderate grade.

Study Period

The study period, comprising the periods for the research study and the compilation of the study literature, was 5 months, from June 2021 to October 2021.

Place of Study

The research study and the compilation of the study literature were done in the Departments of Pharmacology, Clinical Pharmacology, Molecular Pharmacology, Pharmacovigilance, Clinical Medicine, Endocrinology, Pathology, and Clinical Pathology, in Dr. Moumita Hazra's Polyclinic and Diagnostic Centre, Dr. Moumita Hazra's Academic Centre, Dr. Moumita Hazra's Educational Centre, Hazra Nursing Home, Mamata Medical College and Hospitals, Rama Medical College Hospital and Research Centre, Hi-Tech College of Nursing, Shri Ramkrishna Institute of Medical Sciences and Sanaka Hospitals, Raipur Institute of Medical Sciences, Fortis Hospitals, and GIOSTAR IRM Institutes, Hospitals, and Laboratories.

Study Procedure

A total of 250 new early moderate grade type II diabetes mellitus patients were prescribed oral sitagliptin 25 mg once daily or gemigliptin 25 mg once daily for 3 months, in monotherapy, or in combination therapy, or in a mixed regimen of monotherapy and combination therapy, with another oral hypoglycemic agent.

The patients' characteristics, diabetic symptoms assessment, patients' disease, and disease-related history were recorded with a pro forma. Then, thorough general physical examination and systemic examination were performed on the patients under study. The relevant blood, urine, and other investigations were done to confirm the progressing health status of the patients being treated.

The efficacy assessment was done, by recording the fasting and the postprandial blood sugar level, HbA1c level, and urine routine examination findings including sugar and albumin levels and microscopy, at subsequent intervals, and follow-up.

The safety assessment was done by the monitoring of adverse drug reactions, at subsequent intervals, and follow-up.

The prescription patterns of both the drugs were analyzed. The number of prescriptions of 250 patients treated with sitagliptin and gemigliptin was recorded; and the percentage of prescriptions for either drug was calculated.

Statistical Analysis

The respective prescription rates were statistically analyzed by percentages.

RESULTS

The demographic characteristics of the patients were comparable.

Figure 1 depicts that sitagliptin was most commonly prescribed (200 prescriptions, 80%) followed by gemigliptin (50 prescriptions, 20%).

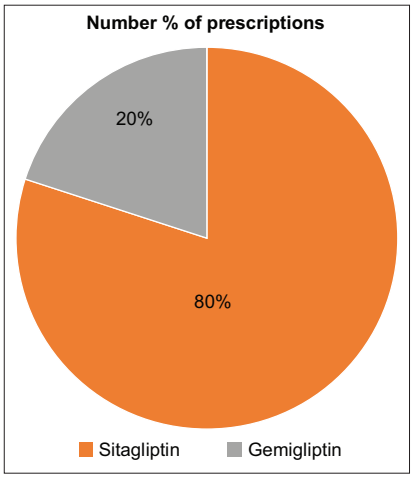

Figure 1: The prescription rates of different anti-diabetic drugs in percentages

The prescription rates of the dipeptidyl peptidase 4 inhibitors were as follows: Sitagliptin > gemigliptin.

The monotherapy, or combination therapy, or mixed regimen of monotherapy and combination therapy of sitagliptin or gemigliptin, with another oral hypoglycemic drug, was observed to be quite efficacious, which had controlled type II diabetes mellitus among new patients, with significant decrease in the blood sugar levels and the HbA1c levels, in the successive 3 months. The adverse effects observed with monotherapy, or combination therapy, or mixed regimen of monotherapy and combination therapy, were statistically non-significant. Therefore, the monotherapy, or combination therapy, or mixed regimen of monotherapy and combination therapy, was safe and tolerable.

DISCUSSION

Diabetes, which is a chronic metabolic disorder, has recently sharply increased on a global scale. According to the International Diabetes Federation (IDF), there were 415 million patients diagnosed with T2D. Diabetes among Asian populations has some distinguishing characteristics from other races in the world, namely, the early decrease in beta-cell function resulting in high postprandial blood glucose and the development to chronic diabetic complications occurs at an early stage of the disease. Hence, a therapeutic agent which increases beta-cell function plays an important role in antihyperglycemic protocols.

Nowadays, anti-DPP4 antihyperglycemic agents have been widely used for patients with T2D under guidelines of diabetes associations and proved to be effective in the enhancement of beta-cell function through ameliorating serum incretin hormone concentrations (two major incretins, GLP-1 and glucose-dependent insulinotropic polypeptide, GIP) – an anti-beta-cell apoptosis agent. There have been two incretin-related therapies for patients with T2D, namely, glucagon-like peptide-1 agonists, exedin-4 and dipeptidyl peptidase-IV inhibitor, sitagliptin. In 2009, the American Association of Clinical Endocrinologists (AACE/ACE) issued the guideline for antihyperglycemic treatment protocol which mentioned about the usage of incretin therapies as the first-line drug for newly diagnosed patients with T2D (i.e., incretin therapies could be monotherapy or in combination with other antidiabetic drugs such as biguanide, sulfonylurea, or insulin). These days, incretin therapies regarding treatment for patients with T2D have been developed on a global scale and shown positive effects on not only glycemic control but also prevention from chronic diabetic

complications as well. While anti-DPP4 agents have many effects on antihyperglycemic conditions, there have been little researches on the Asian population to investigate the role of these drugs on beta-cell function, peripheral insulin sensitivity, insulin resistance, and serum GLP-1 concentrations in comparison to healthy subjects but results were controversial.

In a study, drug choice was based on the guidelines of the American Association of Clinical Endocrinologists and American College of Endocrinology (AACE/ACE 2009). Patients with T2D had low HbA1C concentrations, so sitagliptin was selected as the first choice for treatment therapy in adjunct to lifestyle modification and exercises. The primary endpoint was the change from baseline in GLP-1, HOMA2-B, HOMA2-IR, and HOMA2-S after 3 months of treatment with sitagliptin. Other variables of interest consisted of FPG, lipid profile, safety laboratory measurements (urea, creatinine, ALT, and AST) after 3 months of treatment with sitagliptin. After 3 months of treatment with 100 mg/day sitagliptin, patients illustrated higher HOMA2-B, HOMA2-S, and lower HOMA2-IR to those before interventions. Sitagliptin, one of the anti-DPP4 agents, has been consistently demonstrated to have effects on beta-cell and insulin concentrations indirectly prolonging active incretins and this exhibits L-cells to secrete more GLP-1. Recently, this group of agents was approved to be a second-line therapy for patients with type 2 diabetes mellitus internationally but as recommended by AACE/ACE (2009), the anti-DPP4 agents may be used to start monotherapy for type 2 diabetes patients. One model-based analysis (a placebo-controlled clinical study) found that sitagliptin improved beta-cell function relative to placebo in both fasting and postprandial states in patients with T2D. Although sitagliptin has been shown numerous efficacies in antidiabetic therapy overall, these effects varied from different races. A meta-analysis showed that, among patients with T2D in Asia, sitagliptin had increased insulin sensitivity and weight much higher in comparison to that in the Caucasian population and the between-group (Asia-Caucasian) different in HOMA2-B was - 4.97 (95% CI, - 9.86 to - 0.09, $P < 0.05$). One suggestion for these differences could be due to Asian anthropometric indices including low BMI and high blood glucose due to insulin resistance rather than insulin deficiency. DPP4 inhibitors might induce beta-cell regeneration, prevention from pancreas islet hypertrophy and insulin synthesis *in vitro* studies. DPP4-inhibitors also improved beta-cell function both inside and outside the setting of food consumption, but some studies found that there was no change in the incretin effect. Moreover, DPP-4 inhibitors would allow beta-cells to adapt to the degree of insulin resistance and have a better response to glucose overload and as the result, they decrease the overall insulin exposure and the pro-insulin-to-insulin ratio.[4]

In this study, the demographic characteristics of the patients were comparable. Sitagliptin was most commonly prescribed (200 prescriptions, 80%) followed by gemigliptin (50 prescriptions, 20%). The prescription rates of the antidiabetic drugs were as follows: Sitagliptin > gemigliptin.

The monotherapy, or combination therapy, or mixed regimen of monotherapy and combination therapy of sitagliptin or gemigliptin, was observed to be quite efficacious, which had controlled type II diabetes mellitus among new patients, with significant decrease in the blood sugar levels and the HbA1c levels, in the successive 3 months. The adverse effects observed with monotherapy, or combination therapy, or mixed regimen of monotherapy and combination therapy, were statistically non-significant. Therefore, the monotherapy, or combination therapy, or mixed regimen of monotherapy and combination therapy, was safe and tolerable.[1]

CONCLUSIONS

The prescription frequency of sitagliptin was followed by gemigliptin.

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REFERENCES

1. Hazra M. A study of the usual clinical pharmacological prescription patterns of metformin, sitagliptin, and remogliflozin among the early moderate grade new Type II diabetes mellitus patients in tertiary care hospitals. Int J Sci Stud 2021;9:83-6.
2. Dharmalingam M, Aravind SR, Thacker H, Paramesh S, Mohan B, Chawla M, *et al*. Efficacy and safety of remogliflozin etabonate, a new

sodium glucose cotransporter2 inhibitor, in patients with Type 2 diabetes mellitus: A 24week, randomized, doubleblind, activecontrolled trial. Drugs 2020;80:587-600.
3. Le TD, Nguyen NT, Nguyen ST, Tran HT, Nguyen LT, Duong HH, *et al*. Sitagliptin increases beta-cell function and decreases insulin resistance in newly diagnosed Vietnamese patients with Type 2 diabetes mellitus. Diabetes Metab Syndr Obes 2020;13:2119-27.
4. Solerte SB, D'Addio F, Trevisan R, Lovati E, Rossi A, Pastore I, *et al*. Sitagliptin treatment at the time of hospitalization was associated with reduced mortality in patients with Type 2 diabetes and COVID-19: A multicenter, case-control, retrospective, observational study. Diabetes Care 2020;43:2999-3006.

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Original Article

Correlation between Heart Rate Variability and Pulmonary Function Tests in Tobacco Chewers

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Abstract

Introduction: Tobacco chewing is more prevalent than smoking in India. It causes local lesion such as lichenoid lesions, leukoplakia, and erythroplakia and various cancers of oral cavity, pharynx, larynx, etc. It also adversely affects cardiorespiratory parameters. Studies have shown adverse effects of tobacco chewing and smoking on ventilatory function tests parameters and heart rate variability (HRV). However, there is a paucity of literature on correlation between HRV and pulmonary function test in tobacco chewers. Hence, this study is planned.

Material and Methods: A total of 60 male subjects in the age group of 25–50 years – 30 tobacco chewers and 30 tobacco non-users were included in the study. Subjects with a history of hypertension, diabetes, oral lesion, drug intake, etc., were excluded from the study. Ventilatory function tests were carried out using RMS Med spirometer. HRV was performed by Polyrite 26D.

Results: A negative correlation between FEV_1 and mean HR was seen. There was a positive correlation between FEV_1 and mean RR interval and FEV_1 and LF. The value of correlation coefficient in these parameters was statistically significant. There was a significant negative correlation between FVC and LF.

Conclusion: Correlation exists between few parameters HRV and ventilatory function tests.

Key words: Chewers, Correlation, Heart rate variability, Tobacco, Ventilatory

INTRODUCTION

Tobacco chewing is presumed as non-injurious and considered as an alternative to smoking. However, tobacco chewing has its own health hazards. It is one of the most important risk factors for the development of oral mucosal lesions including various oral pre-cancerous lesions like lichen planus, lichenoid lesions, leukoplakia and erythroplakia. Harmful effects of chewing tobacco on cardiorespiratory system are seldom known. Nicotine present in tobacco is an addictive and cardioactive agent.[1,2] Various studies have shown adverse effects of tobacco chewing and smoking on ventilatory function tests parameters.[3-5] Effect of smokeless tobacco on heart rate variability (HRV) has also been demonstrated. However, there is a paucity of literature on correlation between HRV and pulmonary function test in tobacco chewers.[6-8] Hence, this study was carried.

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MATERIALS AND METHODS

The present study was conducted in the Department of Physiology, Pt. B.D. Sharma PGIMS, Rohtak. A total of 90 male subjects of age group 25–50 years were included in the study. The subjects were divided into three groups. Study was carried out after ethical approval from the Institutional Ethical Committee. Informed consent was obtained from the subjects before proceeding with the procedure. Information was provided in the language familiar to the subjects.

- Group I – 30 male volunteers who were chronic tobacco chewers (non-smokers) for minimum 10 pouch years in continuation with duration of 7 years or more.

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- Group II – 30 male volunteers who had never used tobacco in any form (control group).

Subjects with known history or symptoms of any chronic cardiopulmonary, endocrine, or metabolic disorder, oral lesion, and any drug intake were excluded from the study.

Tests Conducted

1. Ventilatory function tests
2. HRV.

Procedure for Recording Ventilatory Functions

The ventilatory functions were recorded using the RMS Med spirometer. The Med spirometer is an instrument which measures inspiratory and expiratory parameters. The test progress is shown on the computer monitor. The subjects were instructed to apply mouth piece closely to the lips and close their nose with nose clip so as to prevent any leakage of air. Following parameters were recorded:

- Forced expiratory volume in first second (FEV_1)
- Forced vital capacity (FVC)
- FEV_1/FVC%
- Forced expiratory flow rate $_{25-75\%}$ ($FEF_{25-75\%}$)
- Maximum voluntary ventilation (MVV)
- Peak expiratory flow rate (PEFR).

Procedure for Recording FEV_1, FVC, $MEFR_{25-75\%}$, and PEFR

For recording of FVC, FEV_1, $MEFR_{25-75\%}$, and PEFR, the subjects were asked to breathe in and out normally into the mouth piece. Then, the subjects were asked to take deep breath to fill lungs to maximum possible and then exhale into the mouth piece as quickly as possible. All the subjects made three such attempts and the best of them was selected.

Procedure for Recording MVV

For recording of MVV, subjects were asked to inhale and exhale as deeply and quickly for 15 s. Then, MVV was calculated in liters/minute. The subjects were instructed to stop if they felt any discomfort.

Spirometric indices were calculated using best out of three technically satisfactory performances as per recommendations of the American Thoracic Society.[9]

Procedure for Recording HRV

For recording HRV, Digitalized PowerLab 26T Polyrite D was used. Sampling rate was 256 Hz. High and low filters were set at 99 and 0.1 Hz, respectively. The screen sweep speed was kept at 30 mm/s. For R wave detector, channel 3, that is, ECG channel 3 was used. The whole channel was selected for HRV analysis. Position of event is taken as maximum after threshold. Retrigger delay is taken as 0. Ectopics are excluded from the analysis.

Method of Measurement

HRV of subjects was measured with digitalized Polyrite D as per standards laid by Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.[10]

Procedure

The subjects were asked to lie down on the couch and made to relax in front of the Polyrite D system. The three disposable adhesive electrodes were attached to left arm, right leg, and left leg, respectively. The basal recording of ECG (Lead II) was taken for 5 min. From the ECG, the analysis of HRV was done automatically by fast Fourier transformation method.

Outcome of Variables

HRV parameters generated and selected for study.

- Mean heart rate (beats/min)
- Mean RR interval (seconds)
- VLF (ms^2)
- LF (nu)
- HF (nu)
- LF/HF ratio.

Statistical Analysis

All the data obtained by above two procedures were analyzed by a commercially available software package SPSS software. Statistical significance between Group I and Group II was determined using Student's unpaired *t*-test. Pearson's correlation coefficient was used for correlation purpose. $P < 0.05$ was considered statistically significant and $P < 0.001$ was considered highly significant.

RESULTS

Since both the groups were comparable, there was no significant change in terms of anthropometric variations.

All the ventilatory parameters were reduced in Group I and the reduction was significant except for FEV_1/FVC ratio [Table 1]. There was a significant reduction in values of VLF, LF, and HF and a significant increase in LF/HF ratio [Table 2].

There is negative correlation between FEV_1 and mean HR. There is positive correlation between FEV_1 and mean RR interval and FEV_1 and LF. The value of correlation coefficient in these parameters is statistically significant. There is negative correlation between FVC and mean HR. There is very strong negative correlation between FVC and LF [Figure 1]. The value of correlation coefficient in these parameters is statistically significant. There is a significant negative correlation between $FEF_{25-75\%}$ and

Table 1: Ventilatory function tests in Group I and Group II

Parameters	Group I (chewers) (*n*=30) (Mean±SD#)	Group II (control) (*n*=30) (Mean±SD)	*P* value
FEV_1 (L)	1.73± 0.58	2.87± 0.41	0.001*
FVC (L)	1.90± 0.51	3.17± 0.52	0.001*
FEV_1/FVC (%)	90.2±11.4	96.24±11.0	0.84
$FEF_{25-75\%}$ (L/s)	2.31±1.47	3.96±1.06	<0.001**
MVV (L/m)	74.4±30.7	125.9±25.7	<0.001**
PEFR (L/min)	3.53±1.82	7.05±1.98	<0.001**

*P<0.05: Significant, **P<0.001: Highly significant, #SD: Standard deviation. FEV_1: Forced expiratory volume in first second, FVC: Forced vital capacity, $FEF_{25-75\%}$: Forced expiratory flow rate $_{25-75\%}$, MVV: Maximum voluntary ventilation, PEFR: Peak expiratory flow rate

Table 2: Comparison of HRV parameters in Group I and Group II

Parameters	Group I (chewers) (*n*=30) (Mean±SD#)	Group II (control) (*n*=30) (Mean±SD)	*P* value
HR (beats/minute)	80.21±9.56	73.94±15.0	0.059
RR interval (seconds)	744.19±150.66	760.09±93.38	0.628
VLF (ms^2)	501.58±416.83	1977.13±1104.22	0.0001**
LF (nu)	53.72±10.95	59.91±12.87	0.048*
HF (nu)	22.94±6.39	47.07±63.16	0.0001**
LF/HF	2.52±0.91	2.02±0.92	0.035*

*P<0.05: Significant, **P<0.001: Highly significant, #SD: Standard deviation. HRV: Heart rate variability

Table 3: Pearson's correlation between ventilatory function tests and HRV parameters in Group I

Variables	Mean HR	Mean RR	VLF (ms^2)	LF (ms^2)	HF (ms^2)	LF/HF
FEV_1	−0.405*	0.367*	0.058	0.368*	0.078	0.271
FVC	−0.389*	0.273	0.089	−0.68**	0.059	0.281
FEV_1/FVC	−0.130	0.246	0.137	0.039	0.082	−0.217
$FEF_{25-75\%}$	−0.378*	0.403*	0.152	0.298	0.239	0.050
MVV	−0.282	0.282	−0.091	0.252	0.029	0.167
PEFR	−0.344*	0.423*	0.161	0.236	0.156	0.159

*P<0.05: Significant. FEV_1: Forced expiratory volume in first second, FVC: Forced vital capacity, $FEF_{25-75\%}$: Forced expiratory flow rate $_{25-75\%}$, MVV: Maximum voluntary ventilation, PEFR: Peak expiratory flow rate, HRV: Heart rate variability

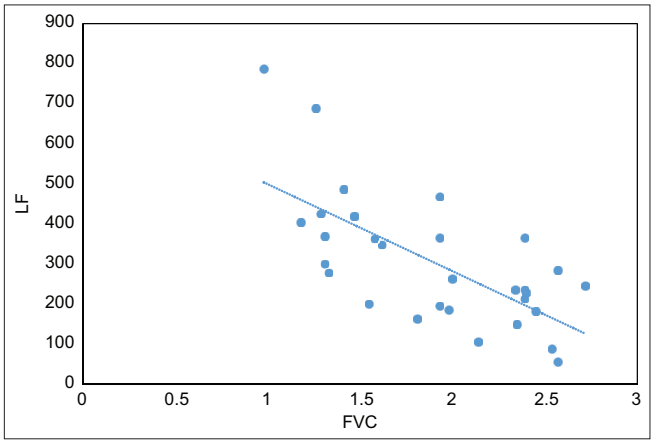

Figure 1: Correlation between LF and FVC in Group I

mean HR, and between PEFR and mean HR. There is a significant positive correlation between $FEF_{25-75\%}$ and mean RR interval and between PEFR and mean RR interval. Correlation coefficient among other parameters is very weak and statistically insignificant [Table 3].

DISCUSSION

Prevalence of smokeless tobacco use is 26% which is far greater than smoking (14%) among adults as reported by Global Adult Tobacco Survey report of India.[11] In our study, all the ventilatory function parameters were significantly reduced in Group I. This was also reported by Pramanik *et al.*[5] Smokeless tobacco products induce oxidative stress resulting from imbalance between formation of reactive oxygen species and antioxidants, contribute to chronic airway limitation.[12] These free radicles alter the cellular antioxidant defense system. Lam *et al.* have demonstrated the release of free radicle nitric oxide from extracts and components of smokeless tobacco in human saliva of SLT users.[13] Some other workers have, however, reported oxygen free radical (O2–) production in cells exposed to smokeless tobacco and nicotine.[13-15] In an animal study, it was demonstrated that long-term (2 weeks) administration of aqueous extracts of gutkha (a type of SLT) rats, impaired the enzymatic antioxidant defense system, and reduces glutathione levels in liver, lung, and kidney, leading to inflammatory changes in these organs.[16]

All the HRV frequency domain parameters except for LF/HF were reduced in Group I, indicating sympathovagal imbalance suggestive of increased vagal tone. Chewing of tobacco results in considerable systemic exposure to nicotine.[17] The predominant cardiovascular effects of nicotine result from activation of the sympathetic nervous system. The state of sympathovagal balance is used for the prediction of many cardiovascular dysfunctions.[14] Studies in both SLT users and smokers have shown cardiac sympathovagal imbalance.[15,16-21] Nicotine increases the cardiac output by increasing both the heart rate and the myocardial contractility.[22]

CONCLUSION

Tobacco chewing affects both HRV and ventilatory function parameters indicating its detrimental effect on both cardiac and respiratory systems. Pulmonary function tests and HRV can be considered as diagnostic tool for preclinical assessment of cardiorespiratory status.

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REFERENCES

1. Williams NS, Bulstrode CJK, O'connell PR. Bailey's and Love's Short Practice of Surgery. 26th ed. Boca Raton: Taylor and Francis Group; 2013.
2. Al-Ibrahim MS, Gross JY. Tobacco use. In: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical and Laboratory Examinations. 3rd ed. Boston: Butterworths; 1990. p. 214-6.
3. Bano R, Mahagaonkar AM, Kulkarni NB, Ahmed N, Nighute S. Study of pulmonary functions among smokers and non-smokers in a rural area. Paravara Med Rev 2009;1:11-6.
4. Nighute S, Tiwari A. A study of the pulmonary function test among smokers and non-smokers in a rural area of Gujarat. J Clin Diagn Res 2011;5:1151-3.
5. Pramanik P. Effect of khaini-a form of smokeless chewing tobacco on pulmonary functions. Indian J Physiol Pharmacol 2013;57:84-6.
6. Glad M, Ratchagan K, Sundaramurthy A. A study of short term heart rate variability in dipping tobacco users. Asian J Med Sci 2013;5:91-4.
7. Pakkala A, Ganashree CP, Raghavendra T. A study of heart rate variability among khaini users: A form of smokeless tobacco in India. Muller J Med Sci Res 2013;4:64-7.
8. Itagi AH, Arora D, Patil NA, Bailwad SA Yunus GY, Goel A, *et al*. Short-term acute effects of gutkha chewing on heart rate variability among young adults: A cross-sectional study. Int J Appl Basic Med Res 2016;6:45-9.
9. Brusasco V, Gapo R, Viegi G. Standardization of spirometry. Series "ATS/ERS task force: Standardization of lung function testing. Eur Respir J 2005;26:319-38.
10. Malik M, Bigger JT, Camm AJ, Kleiger RE, Mallini A, Moss AJ, *et al*. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task force of the European society of cardiology and the North American society of pacing and electrophysiology. Eur Heart J 1996;17:354-81.
11. Ministry of Health and Family Welfare. Global Adult Tobacco Survey India Report. New Delhi: Ministry of Health and Family Welfare, Government of India; 2010. p. 72.
12. Schunemann J, Freudenheim JL, Grant BJ. Epidemiologic evidence linking antioxidant vitamins to pulmonary function and airway obstruction. Epidemiol Rev 2001;23:248-61.
13. Lam EW, Kelley EE, Martin SM, Buettner GR. Tobacco xenobiotics release nitric oxide. Tob induc Dis 2011;1:207-11.
14. Bagchi M, Balmoori J, Bagchi D, Stohs SJ, Chakrabarti J, Das DK. Role of reactive oxygen species in the development of cytotoxicity with various forms of chewing tobacco and pan masala. Toxicology 2002;179:247-55.
15. Wetscher GJ, Bagchi D, Perdikis G, Bagchi M, Redmond EJ, Hinder PR, *et al*. *In vitro* free radical production in rat esophageal mucosa induced by nicotine. Dig Dis Sci 1995;40:853-8.
16. Avti PK, Kumar S, Pathak CM, Vaiphei K, Khanduja KL. Smokeless tobacco impairs the antioxidant defense in liver, lung, and kidney of rats. Toxicol Sci 2006;89:547-53.
17. Benowitz NL, Porchet H, Sheiner L, Jacob P. Nicotine absorption and cardiovascular effects with smokeless tobacco use: Comparision with cigarettes and nicotine gum. Clin Pharmacol Ther 1988;44:23-8.
18. Pal GK, Pal P, Nanda N. Comprehensive Textbook of Medical Physiology. New Delhi: Jaypee the Health Sciences Publisher; 2017. p. 108.
19. Vollset SE, Tverdal A, Gjessing HK. Smoking and deaths between 40 and 70 years of age in women and men. Ann Intern Med 2006;144:381-9.
20. Behera J, Sood S, Gupta R, Kumar N, Singh M, Gupta A, *et al*. Assessing autonomic function in smokers. AMJ 2010;3:712-5.
21. Lucini D, Bertocchi F, Malliani A, Pagani M. A controlled study of the autonomic changes produced by habitual cigarette smoking in healthy subjects. Cardiovasc Res 1996;3:633-9.
22. Gilman SL, Zhou X, editors. Smoke: A Global History of Smoking. London: Reaktion Books; 2004.

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Original Article

Comparative Study of Serum Triglyceride Level in Normal Pregnancy and in Pregnancy with Hypertensive Disorder and its Maternal and Fetal Outcome

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Abstract

Background and Objectives: At present most popular theory is an oxidative stress. Abnormal lipid profiles and reactive oxygen species may have a role in the promotion of oxidative stress and vascular dysfunction seen in pre-eclampsia. To compare and correlate serum triglyceride levels among normotensive and hypertensive disorder in pregnancy.

Methods: This is a prospective comparative study, where data was collected from pregnant women, who were more than 28 weeks of gestation and met the inclusion criteria. Comparison was done on blood pressure, triglyceride level, body mass index (BMI), mode of delivery, maternal and fetal outcome among two groups, i.e., hypertensive and normotensive.

Results: The mean triglyceride level in the hypertensive patient were 183.36 mg/dl with standard deviation (SD) of 53.85 whereas in normotensive serum triglyceride levels were 129.47 mg/dl with SD of 28.08 (*P*-value = 0.00). The mean BMI in hypertensive and normotensive was 24.03 kg/m^2 and 22.94 kg/m^2, respectively.

Conclusion: Levels of serum triglyceride are significantly increased in hypertensive patients. The mean serum triglyceride levels also significantly increased with increase in the severity of hypertension. Hence, it is important to identify the serum triglyceride levels among hypertensive patients, so as to initiate a therapy as soon as possible and curtail the burden of diseases.

Key words: Triglyceride levels, Normal pregnancy, Hypertensive pregnancy

INTRODUCTION

Hypertensive disorders are a long-standing threat that endangers the lives of both mother and child. It can manifest in four forms during pregnancy, i.e., gestational hypertension, chronic hypertension, preeclampsia, and eclampsia. 50–98% of the maternal deaths that occur in India are due to direct obstetric complications which also include hypertensive disorders.[1,2]

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The prevalence of preeclampsia is around 8–10% in India.[2,3] Hypertensive disorders are amongst the top three causes for maternal deaths in India. Being a multisystem disorder, if not identified early, it not only impairs the functions of the kidney and liver but also causes intense vasospasm, HELLP syndrome, postpartum vascular collapse, impaired electrolyte balance, blindness, PRES, etc., effecting almost every organ of the body.[4] Women with pre-eclampsia are also more likely to suffer neonatal death or stillbirth. It might also effect the fetus by causing intrauterine death, intrauterine growth retardation, and prematurity.[5]

Pregnancy is a physiological process that is accompanied by various anatomical, physiological, and biochemical changes of the entire body. During pregnancy total lipid levels increases by 50%. High-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride levels also increase by 15%,

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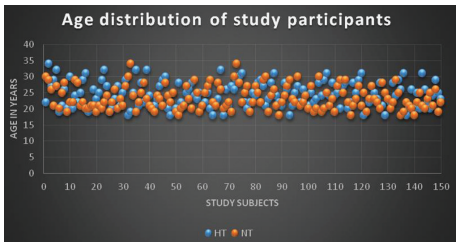
40%, and 50%, respectively. The etiology of pre-eclampsia is still unknown.[2,4,6] Since the etiology and pathogenesis of hypertensive disorders in pregnancy still remains controversial, many markers of endothelial dysfunction have been identified in preeclampsia women. Due to insulin resistance and increased estrogen during pregnancy, there is alteration of circulating triglycerides, fatty acids cholesterol, and phospholipids due to metabolic changes in both the liver and adipose tissue.[7,8] Altered lipid synthesis leads to decrease in PGI2: TxA2 ratio which is also supposed to be an important way of pathogenesis in pregnancy-induced hypertension. As pregnancy continues, this causes hyperlipidemia consisting principally of increased triglycerides.[9,10] Hypertriglyceridemia is one of the potent risk factor for metabolic syndrome. Hence, altered lipid profiles such as increased total cholesterol, LDL cholesterol, triglycerides and decreased HDL cholesterol concentrations are associated with an increased risk of preeclampsia.[11] Hence the current study was undertaken with an aim to show the co-relation between triglyceride levels and the maternal and fetal outcome.

METHODS

A prospective comparative study was done where data was collected from pregnant women, who were more than 28 weeks of gestation and met the inclusion criteria ate and approaching hospital for delivery. Preliminary information regarding the participants was collected after obtaining informed consent by using a questionnaire and the blood samples were collected for the estimation of triglyceride levels. The participant was contacted personally in hospital after delivery to know the outcome of pregnancy.

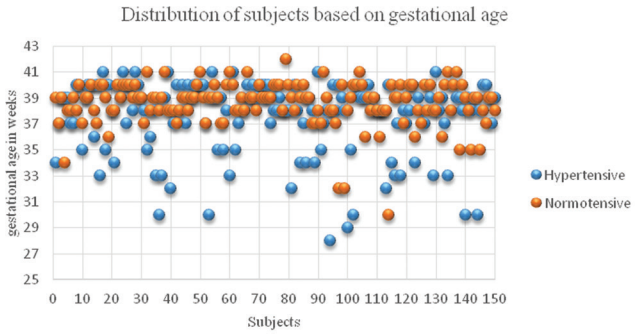
RESULTS

Age distribution among hypertensive subjects varied from 18 years to 34 years. Most of the participants in this group were of 25 years. The mean age was 24.5 years with a standard deviation (SD) of 3.89 years. Age distribution among normotensive subjects varied from 18 years to 34 years. Most of the participants in this group were of 22 years. The mean age was 23.3 years with a SD of 3.59 years. There was no significant difference in the mean age of both the groups, hence both the groups were comparable in terms of age.

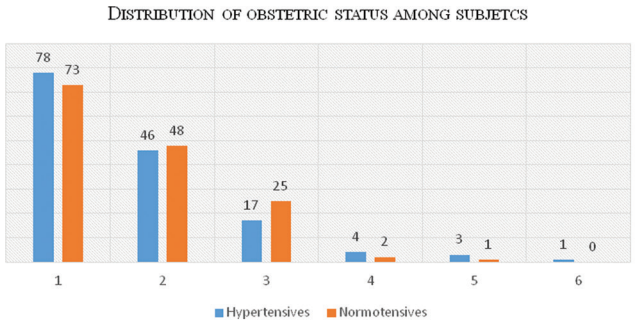


The gestational age among hypertensive patients varied from 28 weeks to 41 weeks. Most of the participants were of 40 weeks of gestation. The mean gestational age in the group was 37.53 weeks with a SD of 2.9 weeks.

The gestational age among normotensives varied from 30 weeks to 42 weeks. Most of the participants were of 39 weeks of gestation. The mean gestational age in the group was 38.56 weeks with a SD of 1.7 weeks. The two groups differed with respect to the gestational age and the difference was statistically significant.

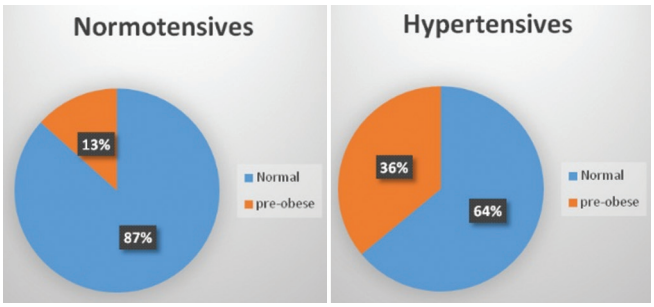


Around 78 participants and 72 participants in the hypertensive group were primigravida and multigravida, respectively. Participants varied from gravida 1 to gravida 6 in this group. The mean of obstetric status with respect to gravida was 1.73 and a SD of 0.982. Around 73 participants and 77 participants in the normotensive group were primigravida and multigravida, respectively. Participants varied from gravida 1 to gravida 5 in this group. The mean of obstetric status with respect to gravida was 1.72 and a SD of 0.836. There was no statistically significant difference between both the groups with respect gravida status, therefore the two groups were similar in terms of their obstetric status.



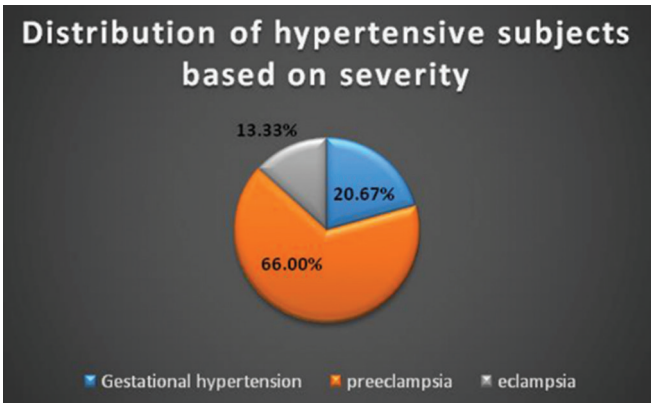
The body mass index (BMI) of the women in hypertensive group varied from 19.20 to 29.3. the mean BMI in the group was 24.03 with a SD of 2.24. About 64% of the women had normal BMI whereas rest 36% were pre-obese. The BMI of the women in normotensive group varied from

19.40 to 26.90. The mean BMI in the group was 22.94 with a SD of 1.65. About 87% of the women had normal BMI, whereas rest 13% were pre-obese. There was significant difference in the BMI of both the groups



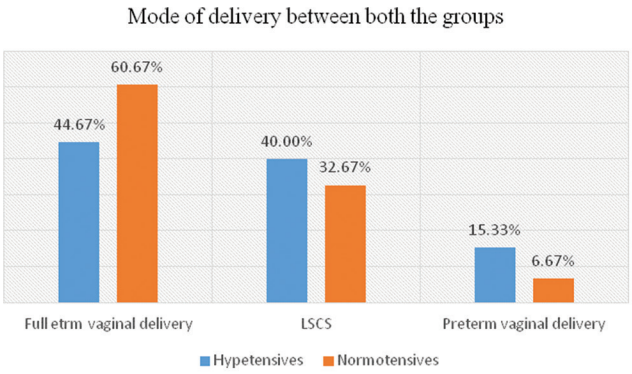
Pre-pregnancy BMI among hypertensive and normotensives

Among the hypertensive group, the mean systolic blood pressure (SBP) was 153.7 with a SD of 10.3. The mean diastolic blood pressure (DBP) in the group was 100.07 with a SD of 8.78. 66% of the subjects had gestational hypertension, 22% had preeclampsia and 13% of them had eclampsia. Among the normotensive group, the mean SBP was 111.6 with a SD of 10.4. The mean DBP in the group was 73.39 with a SD of 8.07. The difference in both systolic and diastolic pressure was statistically significant between both the groups.

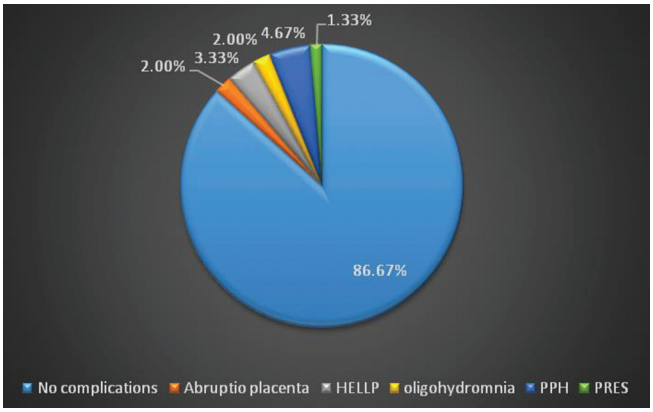


The levels of serum triglyceride varied from 100 to 394 in hypertensive subjects. The mean serum triglyceride levels were 183.36 with a SD of 53.85 in the group. The levels of serum triglyceride varied from 74 to 375 in normotensive subjects. The mean serum triglyceride levels were 129.47 with a SD of 28.08 in the group. The mean serum triglyceride level was significantly higher in hypertensive subjects as compared to normotensive group.

In the hypertensive group out of 150, 67 (45%) of them underwent full-term normal vaginal delivery, 23 (15%) preterm normal vaginal delivery, and the rest 60 (40%) delivered through cesarean section. In the normotensive group out of 150, 91 (61%) of them underwent full-term normal vaginal delivery, 10 (6%) preterm normal vaginal delivery, and the rest 49 (33%) delivered through cesarean section. In both the groups' majority of them underwent normal delivery which were predominantly full term. When compared the frequency of normal delivery was higher in normotensive subjects whereas the frequency of lower segment Cesarian section (LSCS) was higher in hypertensive subjects. The difference in the mode of delivery was statistically significant between both the groups.

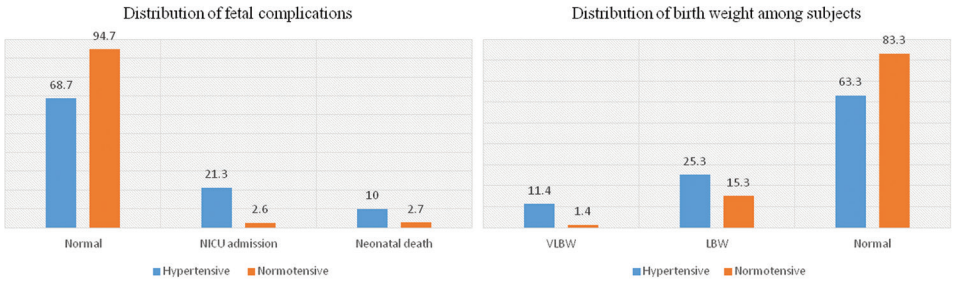


Although 86.67% of the hypertensive subjects did not show any complications, some of the life-threatening complications such as abruptio placenta, HELLP, Oligohydromnia, PPH, and PRES were seen amongst others. Whereas none of the normotensive subjects exhibited any such complications.



The birth weight among normotensive subjects varied from 1 kg to 3.9 kg. The mean birth weight was 2.47 in the group with a SD of 0.66 kg. 83.3% of the babies were of normal weight, whereas 15.3% were low birth weight weighing <2.5 kg but more than 1.5 kg and 1.4% were very low birth weight that is <1.5 kg.

The mean birth weight of the newborns of hypertensive subjects was higher as compared to normotensive and the difference was statistically significant.



In the hypertensive group, 68.7% of the newborns had no complications. 31.3% of the babies were admitted to newborn intensive care unit (NICU) as a result of various complications amongst which 10% died within the first 2 weeks of the postnatal period. In the normotensive group, 94.7% of the newborns had no complications. 5.3% of the babies were admitted to NICU as a result of various complications amongst which 2.7% died within the first 2 weeks of the postnatal period.

The frequency of complications was higher among newborns of hypertensive subjects and the difference of distribution among both groups was statistically significant.

DISCUSSION

Hypertensive disorders are a long-standing threat that endanger the lives of both mother and child. 50–98% of these maternal deaths, that occur in India are due to direct obstetric complications which also include hypertensive disorders.

Age at Pregnancy

In the current study, none of the participants belonged to teenage pregnancy. According to WHO, each year around 21 million girls aged 15–19 years and 2 million girls aged under 15 years become pregnant in developing regions. On the other hand pregnancy over 35 years old, is also associated with increased risks. Older pregnant women demand more information and have anxiety related to the outcome of their pregnancy, the serum triglyceride levels depend on age of the women. It generally increases as the women ages. In the current study, there was no significant difference in the mean age among hypertensive and normotensive women. Hence, both the groups were comparable in terms of age.

Obstetric Status of the Women

Gaillard *et al.*[12] in his study tested the associations between parity and the growth and risk factors in the child. They conducted a population-based prospective cohort study in which fetal and childhood growth were measured on various occassions. Parameters such as body fat distribution, left ventricular mass, blood pressure (BP), blood lipids, and insulin levels at the age of 6 years were also measured. The study found that nulliparous mothers had children with lower risks of preterm birth and small-size-for-gestational-age at birth but a higher risk of large-size-for-gestational-age at birth as compared to multiparous women. Whereas, the multiparous mothers had lower rates of total fat mass percentage, accelerated infant growth, and lower levels of childhood BMI LDL cholesterol than children of nulliparous mothers. Apart from this the children also had clustering of cardiometabolic risk factors. Multiparous women also had a lower risk of childhood overweight and lower cholesterol levels with increasing parity. Therefore, the study stated offspring from nulliparous mothers have lower fetal but higher infant growth rates and higher risks of childhood overweight and adverse metabolic profile.

In the current study, there was no difference in the obstetric status among both the groups. Hence both the groups were comparable in terms of their obstetric status. In both the groups, the number of primigravida and multigravida were almost equal. The obstetric index varied from gravida 1 to gravida 6 in hypertensive subjects and gravida 1 to gravida 5 in normotensive subjects.

Gestational Age

Gestational age at birth is associated with both fetal and neonatal deaths, postnatal death, and morbidities such as the respiratory distress syndrome and necrotizing enterocolitis. Some of the long-term morbidities such as deafness, blindness, hydrocephaly, mental retardation, and cerebral palsy are also manifested.

Though the rate of preterm births decreased, in 2018 preterm birth affected 1 of every 10 infants born in the United States.[13] A developing baby goes through important growth throughout pregnancy - including in the final months and weeks. Pre-term babies have higher rates of death and disability. In 2017, pre-term birth and low birth weight accounted for about 17% of infant deaths. Babies who survive may have[14]

- Breathing problems
- Feeding difficulties
- Cerebral palsy

- Developmental delay
- Vision problems
- Hearing problems.

Pre-term are at an increased risk of cardiovascular disease later in life, including increased mean arterial pressure, abnormally shaped and sub-optimally performing hearts and changes in the vasculature.[14]

In the current study, there were preterm births in both normotensive and hypertensive subjects. The difference in the gestational age group significantly varied in both groups. The mean gestational age among hypertensive subjects was significantly lesser as compared to normotensive subjects.[5,6]

Obesity

The cardio-vascular changes that occur in pregnancy are mainly to attain adequate foeto-placental circulation for the well-being of fetal growth and development. There is vasodilatation of systemic vasculature and the maternal kidneys. Cardiac output increases up to 45%, DBP and SBP decreases.[10] On the contrary some studies also proved increase in BP throughout pregnancy.[11] whereas another population-based study showed an increased BP during pregnancy among obese women.[12]

In short, the cardiovascular system of a pregnant women undergoes significant structural and hemodynamic changes. Apart from increase in cardiac output and a decrease in maternal systemic vascular resistance; it also effects the renin-angiotensin-aldosterone system and the heart and vasculature undergo remodeling. Any maladaptation of these factors has been associated with fetal morbidity. Therefore, understanding the normal cardiovascular changes in pregnancy is essential for the better outcome of pregnancy.

Studies suggest cardiovascular diseases and preeclampsia have certain risk factors which can be identified early.[15] Certain characteristics such as obesity, smoking, psychological stress, and polycystic ovarian disease are consistently associated with preeclampsia. Apart from these certain genetic factors also play a role in enhancing the risk. Certain hormonal disorders, such as polycystic ovarian disease and premature ovarian failure have increased risk for preeclampsia by the fact that these disorders confer increased cardiovascular risk outside pregnancy.[14]

A study done by Gaillard *et al*.,[13] showed that compared to mothers with a normal weight, maternal obesity (BMI 30–34.9 kg/m) and morbid obesity (BMI ≥35 kg/m) were associated with higher first trimester SBP and DBP. The study also showed that these differences were observed during the second and third trimesters also. The risks of pregnancy-induced hypertension and preeclampsia were increased among obese mothers and morbidly obese mothers. Maternal weight gain was associated with the risk of pregnancy-induced hypertension. Maternal obesity was strongly associated with BP in each trimester and increased risks of gestational hypertensive disorders among obese women.[14]

In the current study also there was a significant difference in the BMI of both the normotensive and hypertensive subjects. Although none of the women in either of the groups was obese around one-third of the participants in the hypertensive group were pre-obese.

Pregnancy Induced Hypertension

The prevalence of hypertension was 10% of all pregnancies. Around 7–8% of those who had elevated BP, it was pregnancy-induced ("gestational") without other associated problems. Study found that hypertensive disorders occur in up to 1 in 10 pregnant women.[12] In all the countries, 10% of pregnancies in each of the four countries were identified as hypertensive. The incidence was lowest in Pakistan and highest in Mozambique. Few pregnancies were hypertensive based only on isolated systolic hypertension and some had isolated diastolic hypertension later followed by systolic hypertension. In India, women were diagnosed earlier and had significantly more BP measurements. More than 3/4th of the cases who were hypertensive was non-severe. In India particularly hypertension was diagnosed antenatally and in the mid-late third trimester. Around 40% were diagnosed postpartum. In India, the incidence of postpartum hypertension was lowest, and the timing of diagnosis was median 10 days postpartum, whereas in other countries it was 7 days postpartum.

The incidence of chronic hypertension at <20 weeks gestation was lower in India and Pakistan compared with Mozambique and Nigeria. Most hypertension cases were diagnosed during the antenatal period in India and Mozambique, whereas about half was so diagnosed in Pakistan and Nigeria. The incidence of Pre-eclampsia was same in each of the four countries. Very few women presented with eclampsia as their hypertensive disorder. Overall, chronic hypertension was the rarest (<1.0%), gestational hypertension being the most common (6–12%), and pre-eclampsia intermediate in incidence (3–6%).[14]

Hypertensive disorders in pregnancy are one of the major causes of maternal and prenatal morbidity and mortality.[16-18] Another study done by has found that the prevalence of HDP, gestational hypertension, and preeclampsia are 5.2–8.2%, 1.8–4.4%, and 0.2–9.2%, respectively.[19] The factors that increase the risk of gestational hypertension can be genetic or non-genetic.[20] Some of the factors

such as BMI, anemia, and illiteracy are the important modifiable risk factors. Whereas some other factors like maternal age, primiparous, multiple pregnancies, HDP in previous pregnancy, gestational diabetes mellitus, pre-existing hypertension, pre-existing type 2 diabetes mellitus, pre-existing urinary tract infection, and a family hypertension and some had isolated diastolic hypertension later followed by systolic hypertension.[21,22] In India, women were diagnosed earlier and had significantly more BP measurements. More than 3/4th of the cases who were hypertensive was non-severe. In India particularly hypertension was diagnosed antenatally and in the mid-late third trimester. Around 40% were diagnosed postpartum. In India, the incidence of postpartum hypertension was lowest, and the timing of diagnosis was median 10 days postpartum, whereas in other countries it was 7 days postpartum.[13]

Serum Triglyceride

Study done by Grimes and Wild[23] studied the levels of total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol in women from pre-conception period to postpartum. In the first trimester, there was a decrease in levels of all parameters during the first 6 weeks. As the pregnancy progressed by the 3rd month levels increased and then there was a steady increase throughout pregnancy in the major lipoprotein lipids. By the third trimester, plasma cholesterol levels are 50% higher than routinely seen pre-pregnancy and while triglyceride levels are doubled.[14]

In the current study, the levels of serum triglyceride varied from 100 to 394 in hypertensive subjects. The mean serum triglyceride levels were 183.36 with a SD of 53.85 in the group. The levels of serum triglyceride varied from 74 to 375 in hypertensive subjects. The mean serum triglyceride levels were 129.47 with a SD of 28.08 in the group. The mean serum triglyceride level was significantly higher in hypertensive subjects as compared to normotensive group.

Among the hypertensive subjects, the mean serum triglyceride levels were 157.90 in those who had gestational hypertension, 184.66 in those who had preeclampsia, and 216.40 in those who had ecclampsia. The mean serum triglyceride levels increased with increase in severity of hypertension and this mean serum triglyceride levels varied significantly.

Serum Triglyceride and Maternal Outcome

In the current study both the groups' majority of them underwent normal delivery which was predominantly full term. When compared the frequency of normal delivery was higher in normotensive subjects whereas the frequency of LSCS was higher in hypertensive subjects. The difference in mode of delivery was statistically significant between both the groups. The 86.67% of the hypertensive subjects did not show any complications, some of the life-threatening complications such as abruption placenta, HELLP, Oligohydromnia, PPH, and PRES were seen amongst others. Whereas none of the normotensive subjects exhibited any such complications.

Author conclude that there is elevation of serum lipids among preeclamptic when compared to normal pregnancy. A simple screening test acquired, helps to recognize dyslipidemia in the early second trimester of patients who are at risk of preeclampsia. Due to early detection of altered lipid profile in preeclamptic, the incidence of complications can be decreased, which in turn reduces the materno-fetal morbidity and mortality.

CONCLUSION

The serum triglyceride levels are one of the important indicators of maternal and fetal health status of a pregnant women. The levels of serum triglyceride are significantly increased in hypertensive patients. The mean serum triglyceride levels also significantly increased with increase in the severity of hypertension. Hypertension in pregnancy per se is associated with varied maternal and fetal complications. Hence, it is important to identify the serum triglyceride levels among hypertensive patients, so as to initiate a therapy as soon as possible and curtail the burden of diseases.

REFERENCES

1. Sustainable Development Knowledge Platform; 2019. Available from: https://www.sustainabledevelopment.un.org/SDG3. [Last accessed on 2019 Nov 22].
2. Swain S, Agrawal A, Raghavan S, Oumachigui A, Rajaram P. Maternal mortality in India and strategies for reduction. Int J Gynecol Obstetr 2000;70:D34.
3. Preeclampsia, National Health Portal of India; 2019. Available from: https://www.nhp.gov.in/disease/gynaecology-and-obstetrics/preeclampsia. [Last accessed on 2019 Nov 22].
4. Dutta DC. Textbook of Obstetrics. 8th ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd.; 2015.
5. Spracklen C, Smith C, Saftlas A, Robinson J, Ryckman K. Maternal hyperlipidemia and the risk of preeclampsia: A meta-analysis. Am J Epidemiol 2014;180:346-58.
6. Shen H, Liu X, Chen Y, He B, Cheng W. Associations of lipid levels during gestation with hypertensive disorders of pregnancy and gestational diabetes mellitus: A prospective longitudinal cohort study. BMJ Open 2016;6:e013509.
7. Gallos I, Sivakumar K, Kilby M, Coomarasamy A, Thangaratinam S, Vatish M. Pre-eclampsia is associated with, and preceded by, hypertriglyceridaemia: A meta-analysis. BJOG Int J Obstetr Gynaecol 2013;120:1321-32.
8. Maternal Mortality Ratio (MMR) (Per 100000 Live Births). NITI Aayog; 2019. Available from: https://www.niti.gov.in/content/maternal-mortality-ratio-mmr-100000-live-births. [Last accessed on 2019 Nov 22].
9. Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy. Cardiovasc J Afr 2016;27:89-94.
10. Sanghavi M, Rutherford J. Cardiovascular physiology of pregnancy.

Circulation 2014;130:1003-8.
11. Nama V, Antonios T, Onwude J, Manyonda I. Mid-trimester blood pressure drop in normal pregnancy: Myth or reality? J Hypertens 2011;29:763-8.
12. Gaillard R, Bakker R, Willemsen S, Hofman A, Steegers E, Jaddoe V. Blood pressure tracking during pregnancy and the risk of gestational hypertensive disorders: The generation R study. Eur Heart J 2011;32:3088-97.
13. Thilaganathan B, Kalafat E. Cardiovascular system in preeclampsia and beyond. Hypertension 2019;73:522-31.
14. Herrera E. Lipid metabolism in pregnancy and its consequences in the fetus and newborn. Endocrine 2002;19:43-55.
15. Herrera E, Ortega H. Textbook of Diabetes and Pregnancy. 2nd ed. London: Informa Healthcare; 2008.
16. Mayes P. Lipid transport and storage. In: Murray R, Granner D, Mayes P, Rodwell V, editors. Harper's Biochemistry. 25th ed. Stamford: Appleton and Lange Co.; 2000. p. 268-84.
17. IUPAC commission on the nomenclature of organic chemistry (CNOC)2 and IUPAC-IUB commission on biochemical nomenclature (CBN)3. Eur J Biochem 2008;57:317-8.
18. Wood E. Lehninger's principles of biochemistry 3rd Edition D.L. Nelson and M.M. Cox; Worth, New York, 2000, 1152pp.+Appendices, Â£34.99, ISBN-1-57259-931-6. Biochem Mol Biol Educ 2000;28:286-7.
19. Lampe MA, Burlingame AL, Whitney J, Williams ML, Brown BE, Roitman E, *et al*. Human stratum corneum lipids: Characterization and regional variations. J Lipid Res 1983;24:120-30.
20. Kauffman G. Ullmann's Encyclopedia of Industrial Chemistry Fifth Edition, First International Edition in English by Wolfgang Gehartz, Gail Schulz Thomas Kellersohn, Barbara Elvers, Stephen Hawkins, and Ulrike Winter (Eds.). Chem Educ 2000;5:49-53.
21. White H, Venkatesh B. Clinical review: Ketones and brain injury. Critical Care 2011;15:219.
22. Drummond KE, Brefere LM. Nutrition for Foodservice and Culinary Professionals. 8th ed. United States: John Wiley & Sons; 2014.
23. Grimes SB, Wild R. Effect of Pregnancy on Lipid Metabolism and Lipoprotein Levels. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, et al., editors. Endotext. South Dartmouth, MA: MDText, Inc.; 2000. Available from: https://www.ncbi.nlm.nih.gov/books/NBK498654 [Last accessed on 20 Feb 2018].

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Original Article

Evaluation of Antimicrobial Efficacy of Homeopathic medicines Acid Carbolic, Kreosotum, *Echinacea*, and *Thuja occidentalis* Against *Enterococcus faecalis* Biofilm Formed on Tooth Substrate when compared to 3% Sodium Hypochlorite Solution

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Abstract

Introduction: The usual cause of failure of endodontic therapy is the persistence of microorganisms a major cause being *Enterococcus faecalis*. Sodium hypochlorite, the current gold standard among irrigant, has its own shortcomings and concurrent research for a better antibacterial agent is thus ensued in alternative medicine. The high cure rates of homeopathic medicines in many pathogenic manifestations are evident in literature. When homeopathic medicines are prescribed in ultradiluted form on the basis of symptom similarity, they stimulate body's own defense mechanisms. Hence, homeopathic phytotherapeutic substances which have shown substantial antibiotic, anti-inflammatory, and analgesic effects were tested in this study to assess their antimicrobial potential when compared to sodium hypochlorite.

Materials and Methods: A pure culture of *E. faecalis* was grown on brain heart infusion agar broth and incubated at 37°C overnight. Sixty newly extracted single-rooted teeth were divided into six groups (n = 10) after sectioning to a length of 13 mm. After sterilization, samples were inoculated with bacterial solution and were incubated. Group 1: Distilled water, Group 2: 3% sodium hypochlorite, Group 3: Acid carbolic, Group 4: *Echinacea angustifolia*., Group 5: Kreosotum, At the 2nd and 4th weeks, all groups were treated for 10 min with 3 ml of respective solutions. Quantitative analysis was performed.

Results: Among the tested homeopathic agents, acid carbolic (Group 3) and *E. angustifolia* (Group 4) showed an antibacterial efficacy very much similar to NaOCl in eliminating *E. faecalis* biofilm both at the 2 weeks and 4 weeks.

Conclusion: Homeopathic agents acid carbolic and *Echinacea* can be considered as potential endodontic irrigant.

Keywords: *Enterococcus faecalis*, Biofilm, Acid Carbolic, *Echinacea*, Sodium hypochlorite

INTRODUCTION

One of the cardinal goals of endodontic treatment is root canal disinfection and prevention of ensuing chances of reinfection. As an adjuvant procedure to instrumentation, root canal irrigation strives to eliminate the bacteria from the complex root canal intricacies.[1,2] However, the persistence of the microorganisms in the complex root canal system because of their incomplete elimination from the same due to inefficiency of irrigant can lead to the failure of endodontic treatment.[3,4] *Enterococcus faecalis*, a facultative bacterium, is one of the most prevalently isolated species from failed/infected root canals of both primary and permanent teeth with a prevalence rate of 10–76%.[5-7] An ideal irrigant thus should be able to dissolve necrotic debris, destroy bacteria, lubricate endodontic

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system, and remove the smear layer without affecting healthy tissues.[2,8] Therefore, success of an endodontic therapy largely depends on irrigation of root canal system.

Among the plethora of endodontic irrigant, sodium hypochlorite – NaOCl in the concentration range of 0.5–6% is the most commonly used irrigating solution.[8] However, the untoward effects of NaOCl have also been reported which include an unpleasant taste and odor, inability to abolish the smear layer, toxicity, possible paresthesia of mandibular nerve if extruded into periapical area, allergy, and an increase in coronal microleakage of adhesive restorations.[9-11] This scenario has led to much research in the realm of alternative medicine for an ideal endodontic irrigant.

Thus, homeopathy, one of the alternative medicine systems with its doctrine of "Similia Similibus Curentur," that is, likes are cured by likes and with reported high cure rates in pathogenic manifestations is undoubtedly a viable arena for the search of a better antibacterial agent.[12-16]

Aim

In view with the shortcomings of currently used irrigant in endodontics and the subsequent research for a better agent with higher antibacterial activity and biocompatibility, this study aimed to evaluate the antimicrobial efficacy of acid carbolic, Kreosotum, *Echinacea,* and *Thuja occidentalis* when compared to sodium hypochlorite.

SUBJECTS AND METHODS

For the study, 60 newly extracted non-carious teeth with a single root canal were chosen and divided into six groups (n = 10) for instrumentation. The teeth were immersed in 10% formalin for disinfection and fixation of the organic tissue for a period of 24 h, followed by cleaning of the external debris and calculus with an ultrasonic scaler. Teeth were kept in physiologic saline for the rest of the period. Each tooth was radiographed to establish the presence of a single patent canal, then sectioned with a diamond disc below the cement– enamel junction to achieve a standardized tooth length of 13 mm. The working length of each tooth was determined using a size 10 K-file. Root canals were then instrumented using step-back technique with hand instruments and the canals were enlarged to apical size 40 K-file. Canals were then irrigated with 3 ml of saline during the procedure to remove any debris. The samples were then sterilized by autoclave at 121°C for 20 min.

Bacterial Formation on Tooth Substrate

The specimens were immersed in a 5 ml brain heart infusion (BHI) broth culture of *E. faecalis* (American Type Culture Collection 29212) and cultured for 2 weeks at 37°C with 5% CO_2. Three times a week, the bacterial suspension was replenished with fresh medium, and contamination was tested by Gram staining and replating on blood agar plates. The same process was used to obtain a 4-week bacterial culture [Figures 1 and 2].

Biofilm Verification

Four teeth specimens were examined using a scanning electron microscope at magnification levels of ×1000, ×500, ×5000, and ×10,000 to verify biofilm formation following immersion in 5 ml *E. faecalis* culture and incubation in BHI broth for 2 weeks [Figure 3].

The samples were now divided into six groups based on the irrigant used in each. The irrigation protocol adopted was; irrigation with 15 ml of irrigant, using 30-gauge side vented needle (House Brand –EN122) at a flow rate of 2 ml/min.

Study Groups

Group 1: Distilled water
Group 2: 3% sodium hypochlorite
Group 3: Acid carbolic
Group 4: *Echinacea angustifolia*
Group 5: Kreosotum
Group 6: *T. occidentalis.*

Microbiological Analysis

The root canals were then ground to take out the dentine chips, and using the viable plate count method, colony-forming units (CFUs) were counted and mean CFU was calculated.[17]

Statistical analysis was done using IBM SPSS 20 (SPSS Inc., Chicago, USA). Kruskal–Wallis test with Bonferroni multiple comparison test was applied.

RESULTS

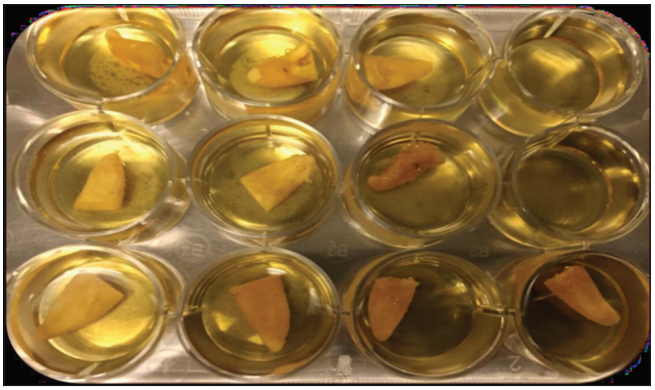


Figure 1: Roots immersed in brain heart infusion broth infected with *Enterococcus faecalis* (ATCC 2122)

Table 1: Number of colonies forming units in 10^6 pre-treatment values of each group

specimens	Group 1 distilled water	Group 2 NaOCl	Group 3 acid carbolic	Group 4 *Echinacea*	Group 5 Kreosote	Group 6 *Thuja*
1	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
2	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
3	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
4	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
5	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
7	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
8	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
9	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
10	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6

Table 2: Mean colony-forming unit values after irrigation at 2 weeks

Irrigant	*n*	Colony-forming unit – 2 weeks			*P* value
		Mean	SD	Median (IQR)	
Group 1 Distilled water	10	5.41	0.09	5.4 (5.3–5.5)	<0.001
Group 2 Sodium hypochlorite 3%	10	1.00	0.00	1.0 (1.0–1.0)	
Group 3 Acid carbolic	10	1.00	0.00	1.0 (1.0–1.0)	
Group 4 Echinacea	10	1.51	0.13	1.50 (1.475–1.525)	
Group 5 Kreosotum	10	4.58	0.43	4.5 (4.2–4.975)	
Group 6 *Thuja occidentalis*	10	4.98	0.31	5.15 (4.65–5.20)	

n: Number of specimens, SD: Standard deviation

Table 3: Mean colony-forming unit values after irrigation at 4 weeks

Irrigant	*n*	Colony-forming unit – 4 weeks			*P* value
		Mean	SD	Median (IQR)	
Group 1 Distilled water	10	5.41	0.09	5.4 (5.3–5.5)	<0.001
Group 2 Sodium hypochlorite 3%	10	1.00	0.00	1.0 (1.0–1.0)	
Group 3 Acid carbolic	10	1.00	0.00	1.0 (1.0–1.0)	
Group 4 *Echinacea*	10	1.62	0.10	1.6 (1.5–1.7)	
Group 5 Kreosotum	10	4.70	0.44	4.5 (4.375–5.125)	
Group 6 *Thuja occidentalis*	10	5.14	0.18	5.2 (4.9–5.3)	

n: Number of specimens, SD: Standard deviation

Pre-treatment values obtained after inoculation with *E. faecalis* are given in Table 1. The results obtained after irrigating the samples with respective irrigant at 2 weeks are summarized in Table 2. The specimens treated with NaOCl showed no bacterial growth. Among the homeopathic irrigant, acid carbolic showed complete elimination of biofilm. *Echinacea* at this point showed significantly less bacterial growth (1.50 × 10 6 CFU/ml). The highest bacterial growth was seen in distilled water (5.41×10^6 CFU/ml) followed by *T. occidentalis* with a mean bacterial colony count of 4.98 × 10 6 CFU/ml followed by Kreosotum with a mean bacterial colony count of 4.58 × 10^6 CFU/ml [Figure 4].

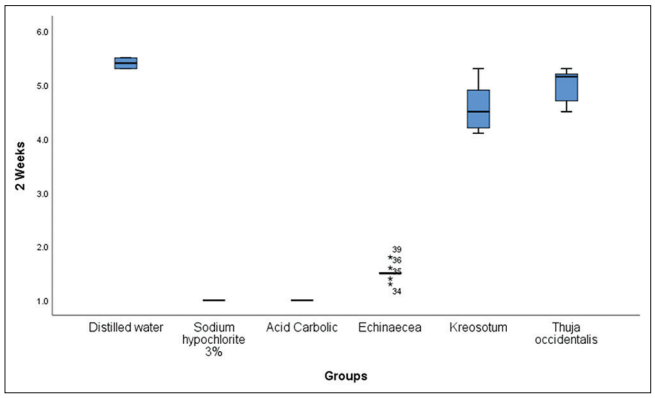
The remaining specimens in each group were similarly processed at the end of 4 weeks of incubation. The values obtained were like those seen at the 2 weeks evaluation. The specimens treated with NaOCl showed no bacterial growth. Among the homeopathic irrigant, acid carbolic showed complete elimination of biofilm. *Echinacea* showed significantly less bacterial growth (1.62×10^6 CFU/ml). The highest bacterial growth was seen in saline (5.41×10^6 CFU/ml) followed by *T. occidentalis* with a mean bacterial colony count of 5.14×10^6 CFU/ml followed by Kreosotum with a mean bacterial colony count of 4.70×10^6 CFU/ml. The results are summarized in Table 3 and Figure 5.

Bonferroni multiple comparison test was done to compare between the values at 2 weeks for each group was done, and it showed that the difference between each group was statistically significant. A similar comparison of the 4th week data also showed statistically significant differences [Graphs 1 and 2].

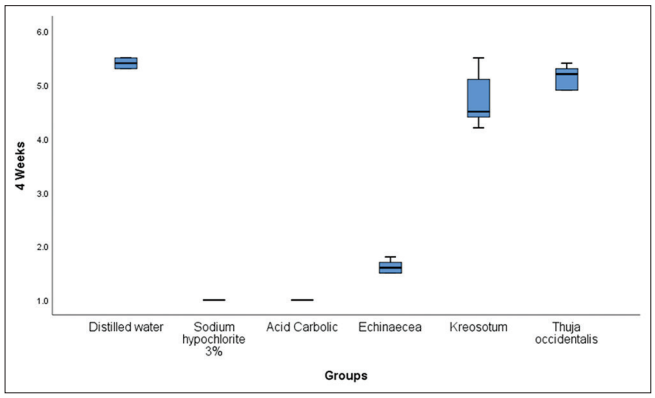
Percentage reduction of bacterial colony count after irrigation both at 2 weeks and 4 weeks period among groups was assessed using multiple comparison test. Statistically significant reduction in colony counts was seen in Group 2 sodium hypochlorite, Group 3 acid carbolic, and Group 4 *Echinacea* at both 2 weeks and 4 weeks. Results of percentage reduction of colony count are depicted in Bar Diagrams 1 and 2.

DISCUSSION

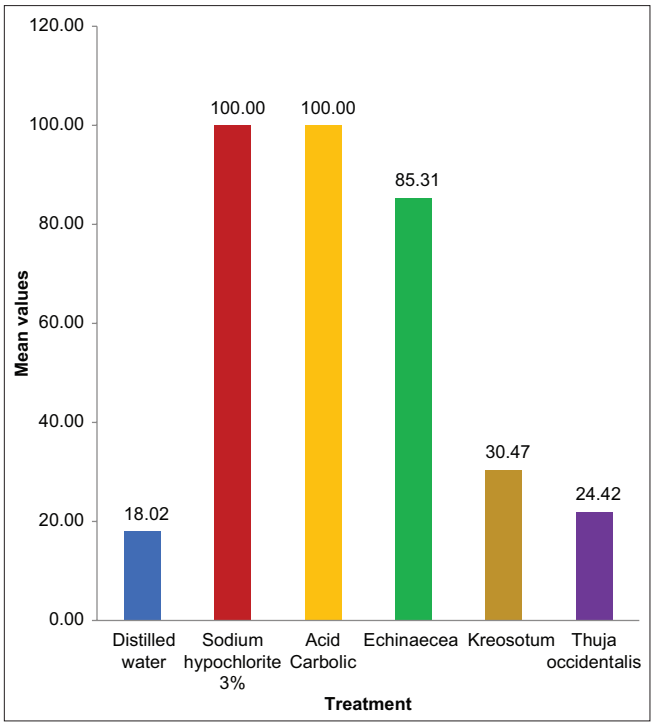
In endodontic therapy, complete control and elimination of infection remains challenging because of various intervening factors. It has been reported that 40% of a root canal system remain untouched even at the completion of cleaning and shaping procedure using rotary instrumentation which harbors bacteria causing reinfection.[18] A major micro organism among these are *E.faecalis* which are isolated from 63% of post treatment



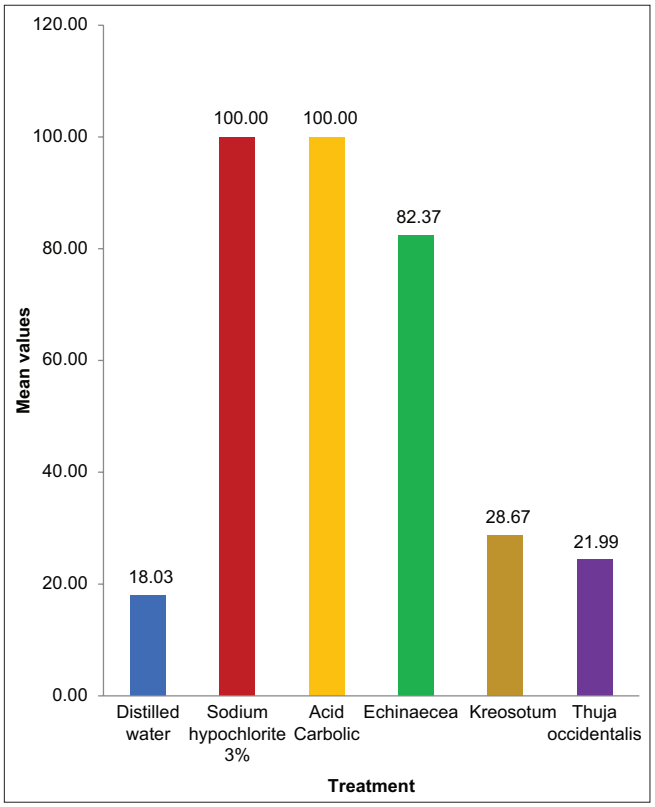
Graph 1: Comparison of colony-forming unit values after irrigation at 2 weeks



Graph 2: Comparison of colony-forming unit values after irrigation at 4 weeks



Bar Diagram 1: Percentage reduction of colony count among groups at 2 weeks



Bar Diagram 2: Percentage reduction of colony count among groups at 4 weeks

diseases. Thus, they entail a major role in etiology of persistent periradicular lesions after endodontic therapy. This underlines the need for endodontic irrigant as inevitable adjuncts to instrumentation in eliminating the microbes, especially in areas which are not accessible to mechanical instrumentation.[19] In the present day, endodontics, a plethora of endodontic irrigant, is being used among which NaOCl recommended as by the American Association of Endodontists as the gold standard for use as an endodontic irrigant.[20] However, as a reason of its high toxicity, corrosive nature, risk of causing emphysema, and severe hypersensitivity reactions, it's use is not approved by FDA.[21] Thus, in retrospection, neither sodium hypochlorite nor any other irrigant currently in use do accommodate the requirements of an ideal root canal irrigant. Thus, many researches have ensued which ponder on considering therapeutic agents in alternative and complementary treatment modalities as viable substitutes considering their minimum adverse effects.[22] Homeopathy is such a complementary medicine system, based on the key principle of "Like cures like" by utilizing phytochemicals and minerals as therapeutic agents. When homeopathic medicines are prescribed in ultradiluted form on the basis of symptom similarity, they stimulate body's own defense mechanisms to fight against pathogens. Homeopathy experiments on basophils and mast cells

showed that homeopathic medicines in ultradiluted form can influence degranulation of mast cells and basophil.[23] In the present study, the antimicrobial efficacy of four different homeopathic medicines when used as endodontic irrigant in the removal of *E. faecalis* biofilms (aged 2 weeks and 4 weeks) from root canals was compared with that of distilled water and 3% sodium hypochlorite. The homeopathic medicines used were acid carbolic, *Echinacea*, Kreosotum, and *T. occidentalis*.

In this study, the representative endodontic pathogen used was *E. faecalis*, because of its reported significance in persistent infections.[24,25] The virulence factor of *E. faecalis* in failed endodontically treated teeth may be related to its capability to invade dentinal tubules and adhere to collagen in the presence of human serum.[26] It can survive harsh environments such as extreme alkaline pH and temperature.[27] It has been reported that the resistance of these microorganisms is increased by the formation of biofilm. Hence, *E. faecalis* biofilm was established on a tooth substrate in this study.[14] Furthermore, a 2-week and 4-week incubation periods were adopted to confirm the biofilm maturation. This is in accordance with a previous report that a dense infiltration reaching 200 microns to 400 microns into the dentinal tubules is achieved during this time frame.[28,29] The present methodology has been adapted from a study by Prabhakar *et al.*[30] and has been corroborated in other similar studies.[31,32]

Being the recommended gold standard among endodontic irrigant, sodium hypochlorite (NaOCl) is capable of promoting biosynthetic changes in cells, metabolic alterations in them, and phospholipid destruction.[33] For killing the bacterial cells in <30 s, studies do report that 3% NaOCl is an efficient concentration.[34] Furthermore, Yesilsoy *et al.* reported that the toxicity of 3% NaOCl is much similar as that of its lesser concentrations.[35] Hence, in this study, 3% NaOCl was used. Although accepted by the American Association of Endodontists for routine use of NaOCl, due to its many short comings including high toxicity, FDA has not approved it.[21] In view with the concerns regarding usage of NaOCl, research in alternative medicine for a suitable irrigant has been the catchphrase of the moment. Homeopathy one of such alternative system has shown potential benefit in treating various diseases of epidemic proportions such as Swine flu, influenza-like illnesses, acute encephalitis syndrome, hemorrhagic dengue fever, diarrheal disorders, and chikungunya with already

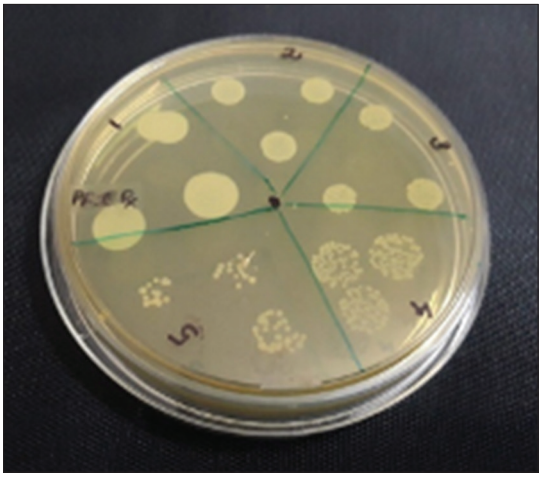


Figure 2: Pre-treatment colony-forming units

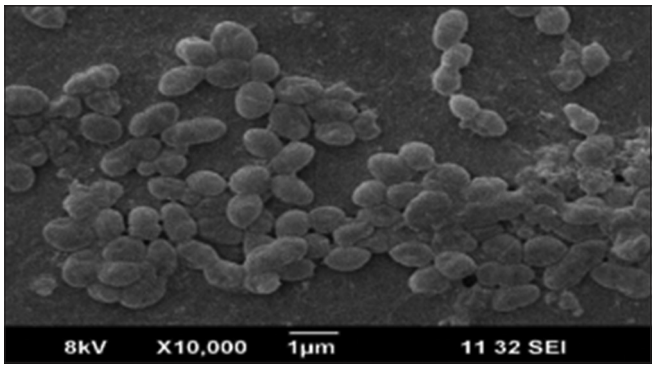


Figure 3: SEM image ×10,000

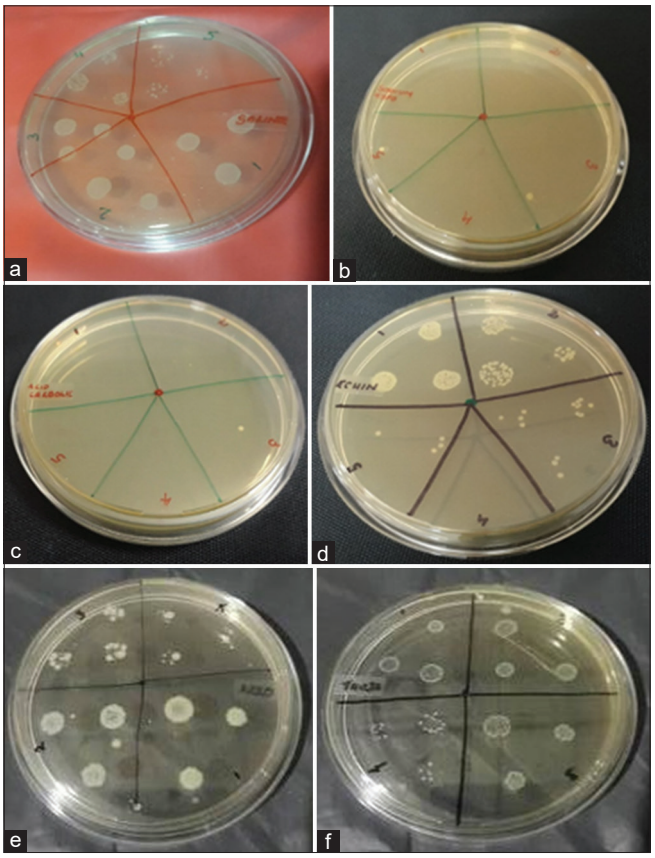


Figure 4: Colony-forming units post-irrigation = 2 weeks, (a) distilled water, (b) sodium hypochlorite (c) acid carbolic, (d) *Echinacea,* (e) Kreosotum, (f) *Thuja*

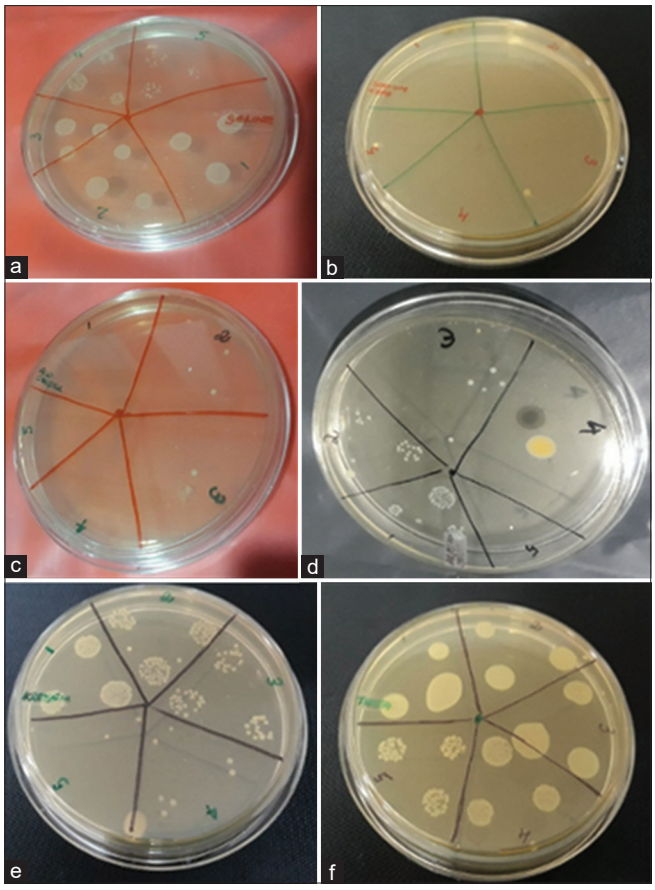


Figure 5: Colony-forming units post-irrigation = 4 weeks, (a) distilled water, (b) sodium hypochlorite, (c) acid carbolic, (d) *Echinacea*, (e) Kreosotum, (f) *Thuja*

existing and approved drugs.[36-44] At present, a previous advisory on the use of Arsenicum album 3°C as an immune booster for prophylaxis against COVID-19 infection is put forward by the Scientific Advisory Board of the Central Council for Research in Homeopathy.[45,46] In the present study, four homeopathic medicines, namely, acid carbolic, *Echinacea*, Kreosotum, and *T. occidentalis* were evaluated.

In the present study, acid carbolic (Group 4) when used as an endodontic irrigant, complete elimination of *E. faecalis* both at 2 weeks and 4 weeks, was observed. The antibacterial action of phenol is long reported in literature to be associated with cytological changes including membrane damage in bacteria.[47] Phenol has adequate penetration power into organic content. It has reported use in infected infections, as in omphalos of neonates. For cutaneous applications for itching, stings, bites, burns, etc., due to its anesthetic action and antibacterial activity to alleviate itching and to manage infections, the use of acid carbolic has been incorporated.[48-56]

Group 4 *Echinacea* showed statistically significant elimination of bacteria both at 2 weeks and 4 weeks. *Echinacea angustifolia* shows antibacterial activity against many microorganisms such as *Pseudomonas* species, *Saccharomyces cerevisiae*, and *Staphylococcus aureus*. A strong growth inhibition activity against *Haemophilus influenzae*, *Legionella pneumophila,* and *Clostridium difficile* has also been reported.[57-59]

In Group 5 (Kreosotum) and in in Group 6 (*T. occidentalis*), statistically significant elimination of bacteria either at 2 weeks or at 4 weeks was not observed. The antibacterial activity of Kreosote and *T. occidentalis* has been reported long back in literature and has a long history of use in treating psoriasis, cystitis, enuresis, uterine carcinomas, bronchial catarrh, amenorrhea and rheumatism.[60-64] The inferior antibacterial activity of Kreosotum agrees with a report by Lucas where Kreosotum extract only showed slight sensitivity against *E. faecalis* (7%) and *E. faecium* (1%).[65] Furthermore, the reduced effectiveness of *Thuja* in our study against a Gram-positive organism like *E. faecalis* can be attributed to the variation in availability of thujone, the major antibacterial component of *Thuja* according to each extraction procedure.[66]

In Group 1 sodium hypochlorite, there was complete elimination of the bacterial biofilm both at 2 weeks and 4 weeks. Elimination of *E. faecalis* can be ascertained to be due to the antibacterial effect of sodium hypochlorite. In Group 2 (distilled water), there was no elimination of *E. faecalis* post-irrigation either at 2 weeks or 4 weeks which can very well be attributed to the ability of *E. faecalis* to survive extreme alkaline pH and salt concentrations.[67-69]

A significant observation from this study is that both the homeopathic medications, namely, acid carbolic and *Echinacea* which were found effective against *E. faecalis* had significant antibacterial efficacy against both 2-week and 4-week biofilms. The fact that antibacterial efficacy of both of these medications was not influenced by the maturation of biofilm is an outcome of this study which is worth pondering

CONCLUSION

Thus, it can be concluded from the findings of the present study that the homeopathic agents acid carbolic and *Echinacea* can be considered as potential endodontic irrigant. An *in vitro* study, by its very nature, is never conducive when compared to that of a clinical scenario. Extensive clinical trials and further research are mandatory before any of these agents can be recognized as total replacements for the conventional and existing irrigant.

REFERENCES

1. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Br Dent J 2014;216:299-303.

2. Topbas C. Endodontic Irrigation Solutions: A Review.
3. Hegde V. *Enterococcus faecalis*; Clinical Significance and Treatment Considerations. Endod 2009;48-52.
4. Frough-Reyhani M, Ghasemi N, Soroush-Barhaghi M, Amini M, Gholizadeh Y. Antimicrobial efficacy of different concentration of sodium hypochlorite on the biofilm of *Enterococcus faecalis* at different stages of development. J Clin Exp Dent 2016;8:e480-4.
5. Jett BD, Huycke MM, Gilmore MS. Virulence of enterococci. Clin Microbiol Rev 1994;7:462-78.
6. Gülhan T, Aksakal A. Virulence factors of *Enterococcus faecium* and *Enterococcus faecalis* strains isolated from humans and pets. Truk J Vet Anim Sci 2006;30:477-82.
7. Ten Cate JM. Biofilms, a new approach to the microbiology of dental plaque. Odontology 2006;94:1-9.
8. Saber SE, El-Hady SA. Development of an intracanal mature *Enterococcus faecalis* biofilm and its susceptibility to some antimicrobial intracanal medications; an *in vitro* study. Eur J Dent 2012;6:43-50.
9. Gernhardt CR, Eppendorf K, Kozlowski A, Brandt M. Toxicity of concentrated sodium hypochlorite used as an endodontic irrigant. Int Endod J 2004;37:272-80.
10. Serper A, Özbek M, Çalt S. Accidental sodium hypochlorite-induced skin injury during endodontic treatment. J Endod 2004;30:180-1.
11. Ehrich DG, Brian JD, Walker WA. Sodium hypochlorite accident: Inadvertent injection into the maxillary sinus. J Endod 1993;19:180-2.
12. Almaguer-Flores A, González-Alva P. Antibacterial activity of homeopathic medications *Lycopodium clavatum* and *Arsenicum album* against periodontal bacteria. Odovtos Int J Dent Sci 2018;20:32807.
13. Poitevin B, Davenas E, Benveniste J. *In vitro* immunological degranulation of human basophils is modulated by lung histamine and *Apis mellifica*. Br J Clin Pharmacol 1988;25:439-44.
14. Sainte-Laudy J, Sambucy JL, Belon P. Biological activity of ultra-low doses I. Effect of ultra-low doses of histamine on human basophil degranulation triggered by *D. pteronissinus* extract. In: Doutremepuich C, editor. Ultra-Low Doses. London: Taylor and Francis; 1991. p. 127-38.
15. Davenas E, Beauvais F, Amara J, Oberbaum M, Robinzon B, Miadonna A, *et al*. Human basophil degranulation triggered by very dilute antiserum against IgE. Nature 1988;30;816-8.
16. Adams J, Tovey P, Easthope G. Mainstreaming Complementary and Alternative Medicine: Studies in Social Context. Milton Park, Abingdon-on-Thames: Routledge; 2017.
17. Pladisai P, Ampornaramveth RS, Chivatxaranukul P. Effectiveness of different disinfection protocols on the reduction of bacteria in *Enterococcus faecalis* biofilm in teeth with large root canals. J Endod 2016;42:460-4.
18. Rahimi S, Janani M, Lotfi M, Shahi S, Aghbali A, Pakdel MV, *et al*. A review of antibacterial agents in endodontic treatment. Iran Endod J 2014;9:161-8.
19. Basrani B. Endodontic Irrigation: Chemical Disinfection of the Root Canal System. Berlin: Springer; 2005.
20. Tosić G, Miladinović M, Kovaević M, Stojanović M. Choice of root canal irrigants by serbian dental practitioners. Vojnosanit Pregl 2016;73:47-52.
21. Segura JJ, Jiménez-Rubio A, Guerrero JM, Calvo JR. Comparative effects of two endodontic irrigants, chlorhexidine Di gluconate and sodium hypochlorite, on macrophage adhesion to plastic surfaces. J Endod 1999;25:243-6.
22. Little JW. Complementary and alternative medicine: Impact on dentistry. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;98:137-45.
23. Gupta VK, Mathur M. Immunomodulatory effects of homoeopathic medicines: A review of pre-clinical studies. Indian J Res Homoeopathy 2018;12:90-4.
24. Nair PR. Pathogenesis of apical periodontitis and the causes of endodontic infection. Crit Rev Oral Bio Med 2004;15:348-81.
25. Graunaite I, Lodiene G, Maciulskiene V. Pathogenesis of apical periodontitis: A literature review. J Oral Maxillofacial Res 2012;2:e1.
26. De Paz LC. Redefining the persistent infection in root canals: Possible role of biofilm communities. J Endod 2007;33:652-62.
27. Radcliffe CE, Potouridou L, Qureshi R, Habahbeh N, Qualtrough A, Worthington H, *et al*. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms *Actinomyces israelii*, *A. naeslundii*, *Candida albicans* and *Enterococcus faecalis*. Int Endod J 2004;37:438-46.
28. Abbaszadegan A, Khayat A, Motamedifar M. Comparison of antimicrobial efficacy of IKI and NaOCl irrigants in infected root canals: An *in vivo* study. Iran Endod J 2010;5:101-6.
29. Kumar TS, Rubin MI, Balaji L, Kandaswamy D. An *in vitro* evaluation of five different herbal extracts as an antimicrobial endodontic irrigant using real time quantitative polymerase chain reaction. J Conserv Dent 2013;16:167-70.
30. Prabhakar J, Senthilkumar M, Priya MS, Mahalakshmi K, Sehgal PK, Sukumaran VG. Evaluation of antimicrobial efficacy of herbal alternatives (Triphala and green tea polyphenols), MTAD, and 5% sodium hypochlorite against *Enterococcus faecalis* biofilm formed on tooth substrate: An *in vitro* study. J Endod 2010;36:83-6.
31. Oncag O, Cogulu D, Uzel A, Sorkun K. Efficacy of propolis as an intracanal medicament against *Enterococcus faecalis*. Gen Dent 2006;54:319-22.
32. Pujar M, Patil C, Kadam A. Comparison of antimicrobial efficacy of triphala, (GTP) green tea polyphenols and 3% of sodium hypochlorite on *Enterococcus faecalis* biofilms formed on tooth substrate: *In vitro* study. J Endod 2010;36:83-6.
33. Hassanshahian M, Bayat Z, Saiedi S, Shiri Y. Antimicrobial activity of *Tachyspermum ammi* essential oil against human bacteria. Int J Adv Biol Biomed Res 2014;2:18-24.
34. Haapasalo M, Orstavik D. *In vitro* infection and disinfection of dentinal tubules. J Dent Res 1987;8:1375-9.
35. Dhulap S, Anita M, Hirwani RR. Phyto-pharmacology of *Elettaria cardamomum*. Pharm Rev 2008;2:27-35.
36. Mathie RT, Baitson ES, Frye J, Nayak C, Manchanda RK, Fisher P. Homeopathic treatment of patients with influenzalike illness during the 2009 A/H1N1 influenza pandemic in India. Homeopathy 2013;102:187-92.
37. Chakraborty PS, Lamba CD, Nayak D, John MD, Sarkar DB, Poddar A, *et al*. Effect of Individualized homoeopathic treatment in influenza like illness: A multicentre, single blind, randomized, place-bocontrolled study. Indian J Res Homoeopathy 2013;7:22-30.
38. Vincent S, Demonceaux A, Deswarte D, Scimeca D, Bordet MF. Management of influenza-like illness by homeopathic and allopathic general practitioners in France during the 2009-2010 influenza season. J Altern Complement Med 2013;19:146-52.
39. Ferley JP, Zmirou D, D'Adhemar D, Balducci F. A controlled evaluation of a homoeopathic preparation in the treatment of influenza-like syndromes. Br J Clin Pharmacol 1989;27:329-35.
40. Oberai P, Varanasi R, Padmanabhan M, Upadhyaya A, Singh S, Singh SP, *et al*. Effectiveness of homeopathic medicines as add-on to institutional management protocol for acute encephalitis syndrome in children: An openlabel randomized placebocontrolled trial. Homeopathy 2018;107:161-71.
41. Nayak D, Chadha V, Jain S, Nim P, Sachdeva J, Sachdeva G, *et al*. Effect of adjuvant homeopathy with usual care in management of thrombocytopenia due to dengue: A comparative cohort study. Homeopathy 2019;108:150-108
42. Jacobs J, Jiménez LM, Malthouse S, Chapman E, Crothers D, Masuk M, *et al*. Homeopathic treatment of acute childhood diarrhea: Results from a clinical trial in Nepal. J Altern Complement Med 2000;6:131-9.
43. Jacobs J. Homeopathic prevention and management of epidemic diseases. Homeopathy 2018;107:157-60.
44. Wadhwani GG. Homeopathic drug therapy homeopathy in chikungunya fever and post-chikungunya chronic arthritis: An observational study. Homeopathy 2013;102:193-8.
45. Azis SP, Kaur H. Enabling use of Homoeopathy in India's preparedness for pandemic/epidemic situation like COVID-19. Indian J Res Homoeopathy 2020;14:143-51.
46. Guidelines for Homoeopathy Practitioners for Prophylaxis and Symptomatic Management of Home IsolatedCOVID-19 Patients; 2019.
47. Corre J, Luccmni J, Mercmr G, Cremieux A. Antibacterial activity of phenol and resulting membrane alterations. Res Microbiol 1990;141:483-97.
48. Maleš Ž, Bojic M. Antioxidant potential of polyphenols: In need for critical assessment of *in vitro* results. J Pharmacogn Nat Prod 2016;2:e104.
49. Bouyahya A, Abrini J, Baaboua A, Bakri Y, Dakka N. Determination of phenol content and antibacterial activity of five medicinal plants ethanolic extracts from North-West of Morocco. J Plant Pathol Microbiol 2016;7:342.
50. Rosa LS, Silva NJ, Soares NC, Monteiro MC, Teodoro AJ. Anticancer properties of phenolic acids in colon cancer a review. J Nutr Food Sci 2016;6:468.
51. Maize KA. *Zea mays* L. genotypes differ in phenology, seed weight and

quality protein and oil contents when applied with variable rates and source of nitrogen. J Plant Biochem Physiol 2016;4:164.
52. Karar MG, Quiet L, Rezk A, Jaiswal R, Rehders M, Ullrich MS, *et al*. Phenolic profile and *in vitro* assessment of cytotoxicity and antibacterial activity of *Ziziphus spina*-christi leaf extracts. Med Chem 2016;6:143-56.
53. Kurata R, Adachi M, Yamakawa O, Yoshimoto M. Growth suppression of human cancer cells by polyphenolics from sweet potato (*Ipomoea batatas* L.) leaves. J Agric Food Chem 2007;55:185-90.
54. Jaganathan SK. Growth inhibition by caffeic acid, one of the phenolic constituents of honey, in HCT 15 colon cancer cells. Sci World J 2012;2012:372345.
55. Wang D, Xiang DB, He YJ, Li ZP, Wu XH, Mou JH, *et al*. Effect of caffeic acid phenethyl ester on proliferation and apoptosis of colorectal cancer cells *in vitro*. World J Gastroenterol 2005;11:4008-12.
56. Gocho S. Antibacterial mechanism of aroma chemical vapors. J Antibacterial Antifungal Agents 1991;19:511-3.
57. Inouye S, Takizawa T, Yamaguchi H. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J Antimicrob Chemother 2001;47:565-73.
58. Stoll A, Renz J, Brack A. Isolation and constitution of the echinacoside, a glycoside from the roots of *Echinacea austifolia.* D.C. Helv Chim Acta 1950;33:1877-93.
59. Cruz I, Cheetham JJ, Arnason JT, Yack JE, Smith ML. Alkamides from *Echinacea* disrupt the fungal cell wall-membrane complex. Phytomedicine 2014;21:435-42.
60. Verma P, Vaid I. Encyclopaedia of Homeopathic Pharmacopoeia. Vol. IB. New Delhi, India: Jai Publishers; 2002.
61. Von Keller G, von Keller G. Symptom Ensa Mmlungen Hom? Opathischer Arzneimittel: Kreosotum, Haug; 1973.
62. Ogata N, Baba T. Analysis of beechwood Creosote by gas chromatography mass spectrometry and high perfomance liquid chromatography. Res Commun Chem Pathol Pharmacol 1989;66:411-23.
63. Tsiri D, Graikou K, Pobłocka-Olech L, Krauzebaranowska M, Spyropoulos C, Chinou I. Chemo systematic value of the essential oil composition of *Thuja* species cultivated in Poland antimicrobial activity. Molecules 2009;14:4707-15.
64. Gershenfeld L, Pressman R, Wood HC Jr. The bactericidal efficiency and toxicity of creosote and its components. J Am Pharm Assoc 1933;22:198-204.
65. Lucas EH, Lewis RW. Antibacterial substances in organs of higher plants. Science 1944;100:597-9.
66. Bell IR, Schwartz GE. Enhancement of adaptive biological effects by nanotechnology preparation methods in homeopathic medicines. Homeopathy 2015;104:123-38.
67. Hancock LE, Gilmore MS. Pathogenicity of enterococci. In: Fischetti VA, editor. Gram-Positive Pathogens. American Society for Microbiology, Washington, DC: 2000. p. 251-8.
68. Facklam RR, Carvalho GS, Teixeira LM. History, taxonomy, biochemical characteristics and antibiotic susceptibility testing of Enterococci. In: Gilmore MS, editor. The Enterococci: Pathogenesis, Molecular Biology and Antibiotic Resistance. Washington, DC: ASM Press; 2002. p. 4-7.
69. Gilmore MS. The Enterococci: Pathogenesis, Molecular Biology, and antibiotic Resistance.Washington, DC: ASM Press; 2002.

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