

eCommons@AKU

Section of Dental-Oral Maxillofacial Surgery

Department of Surgery

January 2018

Evaluation of micro leakage of root canals filled with different obturation techniques: an in vitro study

Maham Muneeb Lone Jinnah Sindh Medical University, Karachi, Pakistan.

Farhan Raza Khan The Aga Khan University, farhan.raza@aku.edu

Follow this and additional works at: https://ecommons.aku.edu/ pakistan_fhs_mc_surg_dent_oral_maxillofac

Part of the <u>Dentistry Commons</u>

Recommended Citation

Lone, M., Khan, F. R. (2018). Evaluation of micro leakage of root canals filled with different obturation techniques: an in vitro study. *Journal of Ayub Medical College, Abbottabad,* 30(1), 35-39. **Available at:** https://ecommons.aku.edu/pakistan_fhs_mc_surg_dent_oral_maxillofac/45

ORIGINAL ARTICLE EVALUATION OF MICRO LEAKAGE OF ROOT CANALS FILLED WITH DIFFERENT OBTURATION TECHNIQUES: AN IN VITRO STUDY

Maham Muneeb Lone, Farhan Raza Khan*

Department of Operative Dentistry, Jinnah Sindh Medical University, Karachi, *Department of Operative Dentistry, Aga Khan

University, Karachi-Pakistan

Background: Despite a plethora of studies done comparing different obturation techniques using gutta-percha, there is no consensus as to which obturation technique results in a 'better' sealing of root canal space. Aims of the study are to compare mean apical micro leakage in root canals of extracted teeth obturated with cold laterally compacted gutta-percha and thermoplasticised injectable gutta-percha using calcium hydroxide based sealer. Methods: It was an in-vitro experimental study carried out using extracted teeth. After access cavity preparation and canal preparation in 70 teeth, they were randomly divided into 2 groups and filled with two different obturation techniques using Sealapex sealer. Teeth were placed in 2.0% methylene blue solution, sectioned longitudinally, observed under microscope and images were captured using microscope attached camera. Amount of dye penetration was measured in millimetre from apex to most coronal part of dye penetration. Data was analysed using SPSS 20.0. Mean and standard deviation of continuous variables was computed. Independent Sample t- test was applied to compare micro leakage values in the two study groups. Level of significance was kept at 0.05. Results: According to the measurements, obturation with Obtura-II and Sealapex was leakier than the other group, with a mean dye penetration of 1.91±1.15 mm. There was a statistically significant difference in dye penetration among the two groups. Conclusions: Cold lateral compaction plus Sealapex was the best combination for obturation as it exhibited least microleakage. Obtura II-Sealapex combination should be used with caution as it showed maximum microleakage. For obturation of single rooted teeth, we recommend cold lateral condensation with Sealapex sealer as it showed better sealability. Obtura-II and Sealapex should be used with caution in single rooted teeth as this group showed the maximum leakage.

Keywords: Dye penetration; Endodontics; Obturation

Citation: Lone MM, Khan FR. Evaluation of micro leakage of root canals filled with different obturation techniques: An in vitro study. J Ayub Med Coll Abbottabad 2018;30(1):34-9.

INTRODUCTION

A well-established primary reason for pulpal and periapical pathology is said to be the presence of bacteria within the root canal space of the tooth.^{1.2} An ideal root canal filling should seal all portals of exit to the periodontium, be well condensed throughout the length of the root canal space and be in close adaptation to the walls of the canal. Apical seal is considered to be the most crucial factor for the success of a root canal treatment. Dow and Ingle suggested that 60% of root canal treatment failures can be attributed to re-entry of microorganisms from the peri-radicular area into the incompletely obturated root canals.³ Hence, the apical sealing property of root canal filling materials is considered the most essential when obturating a root canal space.

'Hermetic' seal has been used traditionally to define the seal provided by root canal obturation materials, which in its literal sense means 'sealed against entry or escape of air'. Keeping this definition in mind, bacteria- tight or fluid impervious seal is used more often in contemporary literature when explaining seal provided by obturation materials.⁴ Most obturation techniques employ a sealer and a core material in an attempt to provide this 'fluidimpervious' seal. Despite extensive research done, there is still no one technique that has shown creation of a complete seal.

Microleakage, in reference to endodontics, is defined as the clinically undetectable passage of bacteria, ions, molecules and fluids along the interface of the obturation material and the prepared root canal dentinal walls or through voids within the obturation materal.⁵ One of the most commonly used methodology for micro-leakage evaluation is based on dye penetration, where after the obturation of the cleaned root canal space, the specimen is immersed in a suitable solution for a specified period of time.^{3,6–}

⁸ Even though many different solutions have been used in research studies to assess micro-leakage, methylene blue has been the most widely used to assess the dye penetration as a measure to quantify microleakage. Different concentrations and varying times of immersion of these dyes have been reported in numerous studies that have observed the dye penetration to evaluate microleakage. Gutta percha has been one of the most commonly used core obturation material for decades by clinicians.⁹ It is available to the dentist in the α -form (gutta percha pieces used in thermoplasticised form for warm condensation) or the β -form (gutta percha points used for cold condensation obturation). Wide arrays of sealers are available in the market with varied compositions for use along with the gutta percha. The sealer is needed to effectively fill in the voids between the gutta percha and flow into the root canal ramifications i.e. lateral and accessory canals where the gutta percha is unable to reach.¹⁰ Calcium hydroxide based sealers are used because of their known antimicrobial properties. Despite a plethora of studies carried out internationally, and a few local studies, conflicting results are seen regarding the sealability of different obturation materials and techniques, with no consensus as to which one is superior to the other.

The aim of our study was therefore to determine which obturation technique using gutta percha as the core filling material and Sealapex as a sealer would best seal the tooth- obturation material interface and therefore, increase the chances of success of a root canal treatment. These results will in turn help the clinician in formulating clinical recommendations for obturation of the root canal space.

The aim of the present study was to compare the mean apical microleakage in root canals of extracted teeth obturated with cold laterally compacted gutta percha and thermoplasticised injectable gutta percha using calcium hydroxide based scaler.

MATERIAL AND METHODS

It was an in-vitro, experimental study carried out at Dental Clinics, Laboratory and Juma Building Research Laboratory of Aga Khan Universty Hospital, Karachi.

Extracted single rooted maxillary teeth (central incisor, lateral incisor, canine) and extracted single rooted mandibular teeth (central incisor, lateral incisor, canines and 1st and 2nd premolars) were included in the sample.

Teeth with cracks, root caries, internal/ external resorption, immature apices, excessive curvatures and previously root canal treated were excluded from the sample.

There were 35 teeth in each group. Since, we had two groups; the total sample size was 70 teeth for this study.

The study was approved by the AKUH ethical review committee before commencement (3271-Sur-ERC-2014). All procedures were completed by one investigator. All extracted teeth satisfying the inclusion criteria were retrieved from the 'tooth-bank' maintained at the AKUH Dental Clinic where teeth extracted for orthodontic or periodontal reasons had been stored. The teeth were cleaned with scaler to remove all debris, disinfected with 5.25% NaOCl and stored in normal saline until experimentation.

Standard access openings were prepared ensuring that straight line access was established to the canal space. Patency of root canals was established and glide path made by using K- files. The working length was determined by the 15 K file kept 1 mm short of the apex. Root canal preparation was done using ProTaper rotary system following the recommended sequence (S1, S2, F1, F2 and F3). RC prep (17.5% EDTA and 10 % urea peroxide) was used as a lubricant and to help in removal of smear layer and inorganic debris. 5.25% Sodium Hypochlorite was used as an irrigant to flush the canal clean of dentinal chips and remove the organic debris. The canals were also thoroughly flushed with distilled water after each use of Sodium hypochlorite.

Once all teeth of the sample had been prepared, they were dried with paper points and then randomly divided into 2 groups and obturated with gutta-percha as follows:

Group I: Using cold lateral condensation (CLC) technique and Sealapex sealer, n=35

Group II: Using Obtura-II and Sealapex sealer, n=35.

When using cold lateral condensation technique, ProTaper gutta percha point that fitted to within 0.5 mm of the working length with tug back, was used as the master cone. Freshly mixed sealer was coated around the master gutta percha point and it was then placed with in the root canal space. Lateral compaction was done and accessory cones with light coats of sealer around them were placed till the spreader penetrated no more than 2 mm into the canal space. Excess gutta percha from the canal orifice was removed by using Touch 'n Heat and then vertically condensed with endodontic pluggers to the level of the canal orifice.

When obturating a canal with the thermoplasticised gutta percha, freshly mixed sealer was coated onto the canal walls by the help of a paper point put to length. A 23-gauge needle on to which a rubber stopper at 4-5mm short of working length was placed was selected and placed into the Obtura-II gun. A new pellet of gutta percha was placed into the gun before the start of each obturation. Once the temperature of the Obtura-II unit reached 200 °C, as confirmed by the display, the needle was placed into the canal and 3–4 mm of gutta percha was expressed into the canal passively. Vertical pressure was then applied with the help of endodontic plugger for compaction of the gutta percha in the apical area.

Successive increments of 3-4 mm each were placed, with compaction done after every increment to compensate for the shrinkage of the gutta percha on cooling. Excess gutta percha in the pulp chamber was removed by the Touch' N Heat device.

After sealing the access cavities using Cavit, teeth were placed for 7 days at 37 °C and 100% humidity to ensure complete setting of the sealer. After 1 week, teeth were air dried and covered with 2 layers of nail polish, except 1-2 mm around the apex. All specimens were immersed in 2.0% methylene blue at 37 °C for 10 minutes, after which they were washed and dried (Figure-1). The teeth were then sectioned longitudinally in a buccolingual direction using a slow speed diamond saw. The split segments were examined using a light microscope (magnification X4) to assess the dve penetration from the apex to the most coronal part of the root (Figure-2). This distance was measured in millimetres on digital images of sectioned specimens which had been captured using a microscope attached camera.

Data was analysed using SPSS 20.0. Frequency distributions of categorical variables, i.e., arch type and tooth type was determined. Mean and standard deviation of continuous variables i.e. length of root in mm, micro leakage values in millimetres was computed.

Independent Sample *t*-test was applied to compare micro leakage values in the two study groups. Level of significance was kept at 0.05.

RESULTS

Each experimental group had 35 specimens, giving a total of 70 readings as the section with the greatest dye penetration was assessed.

There were a total of 24 maxillary and 46 mandibular teeth in the study sample. The mean length of the maxillary teeth was 21.98±2.28 mm, while that of the mandibular teeth was 19.80±1.82 mm

As described in table-1; mandibular teeth showed greater mean dye penetration compared to maxillary teeth. Mean apical dye penetration for the maxillary teeth was 1.70±1.04 mm and for mandibular teeth was 1.52+1.01 mm

Table-2 describes the magnitude of dye penetration measured in the two experimental groups. The mean micro leakage values recorded were highest for the Obtura II and Sealapex sealer group. i.e., 1.91±1.15 mm, while that of the Cold lateral condensation and Sealapex sealer group was 1.25±0.74 mm. Result of independent sample t- test showed a statistically significant difference between the study groups.

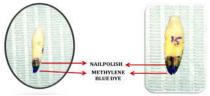


Figure-1: Specimen after application of two coats of nail polish and placement in methylene blue dye

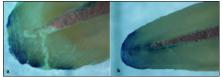


Figure-2: (a, b) Digital image of specimen in group I (cold lateral condensation and sealapex) and group II (obtura-II and sealapex) as seen under the microscope

Table-1: Apical dye penetration in millimeters according to arch type									
Study Groups	n	Minimum (mm)	Maximum (mm)	Mean (mm)	Standard Deviation				
Maxillary	24	0.55	4.40	1.70	1.04				
Mandibular	46	0.00	3.97	1.52	1.01				
Total	140	0.00	4.40	1.58	1.01				

Table-2: Comparison of dye penetration in the experimental groups									
Study Groups	n	Minimum (mm)	Maximum (mm)	Mean (mm)	Standard Deviation	p-value			
CLC & Sealapex	35	0.51	3.97	1.25	0.74	0.002*			
Obtura II & Sealapex	35	0.00	4.40	1.91	1.15	0.002			

Student's *t*- test was applied. $p \leq 0.05$.

DISCUSSION

In the present study, technique of dye penetration with methylene blue dye was used to compare apical leakage in obturated root canals. A number of techniques have been reported in literature to assess the microleakage of obturated root canal space. These include use of scanning electron microscopy,

bacterial penetration, radioisotope penetration, electrochemical evaluation, fluid filtration and dye penetration. According to Wu et al.11, dye penetration or radioisotope penetration has been used in as many as 82% of leakage studies conducted in endodontics. Dye penetration tests are favoured as they are cost effective and easier to perform with minimum armamentarium. The use of 2.0% methylene blue has been the concentration mostly employed in leakage studies. At this concentration it is detectable under visible light, is water soluble, easily diffusible but at the same time no uptake by the dentine matrix is observed.¹² Keeping this in mind, we also used methylene blue to assess the microleakage in our study. Methylene blue was also the preferred choice because it has a molecular size that is comparable to a few bacterial by-products, e.g. butyric acid, that has been reported to leak from the infected root canal space into the periapex, causing irritation in the periapical tissues.^{13,14}

The extent of penetration of the dye from the apex coronally, indicated the seal formed at the obturation material and root dentin interface. This was measured in millimetres by calibrating digital images captured through the microscope via a camera that was attached to it. Similar methodology for measuring the dye penetration has been carried out in numerous studies.^{7,13,15–18} A slight variation to the methodology has also been proposed and carried out with success where after dye immersion, authors have treated the teeth with nitric acid to demineralize the root, achieving transparency and thus allowing a better three dimensional assessment of dye penetration.^{8,10,19–21}

In order to mimic the root canal treatment procedure as carried out in-vivo; all the steps from access cavity preparation to obturation of the root canal space were kept similar to that in clinical settings. Only teeth with single roots and single patent root canals were included in the study so that any anatomical variation could be minimized and a standard comparison be done. To minimize confounding factors, all canal preparations were carried out to the same final apical size. Also, all procedures were done by a single operator. Teeth with excessive curvature were excluded from the study sample as difficulty was anticipated to maintain uniformity during longitudinal sectioning of such roots.

The use of α-gutta percha for thermoplasticised obturation has gained popularity over the years because it circumvents the inherent problems discussed in literature when using the said 'gold standard' cold lateral condensation technique. Compaction of thermoplasticised gutta percha is said to create a more homogenous root canal filling with less voids seen in the gutta percha mass that are otherwise created because of spreader use and the inherent non-adhesive nature of β-gutta percha points when using cold lateral condensation obturation technique. The Obtura-II system is а thermoplasticised gutta percha system in which gutta percha pellets are put in a delivery gun and expressed into the root canal space once heated to a temperature

of 200 °C. To compensate for the shrinkage associated with cooling of the gutta percha that would clinically manifest as voids in the obturation material on radiographic examination, compaction after 3–4 mm of incremental placement of gutta percha is advocated before subsequent increment is injected.²² This recommended protocol was followed in the present study to prevent formation of voids that may otherwise result from the contraction of α -gutta percha on cooling.²³

After obturation, the teeth were placed at 37 °C in 100% humid environment for a period of seven days. These conditions are recommended for complete setting of sealer and to provide an environment that mimics the oral cavity as closely as is possible in experimental settings.^{67,12,24}

A study by Rahimi *et al.*⁷ compared apical microleakage following canal obturation with cold lateral and thermoplasticised gutta-percha compaction techniques had results similar to the present study. The mean dye leakage was more in Obtura-II group compared to leakage in cold lateral condensation group. The values measured in the said study were 3.04 ± 1.42 mm and 2.58 ± 1.09 mm respectively. The authors reported no statistically significant difference between the groups which is in contrast to our study where the two study groups had a statistically significant difference.

The Obtura-II group was the leakiest out of the two groups, mean leakage of 1.91 mm, with a maximum of 4.40 mm. This high value can be attributed to the phase changes brought about by heating the gutta percha, which leads to transformation of crystalline phase of gutta percha to its amorphous phase. The original phase is only regained if the gutta percha undergoes very slow cooling (0.5 °C per hour).^{23,25,26} Nonetheless, in clinical settings, β -phase is said to reform since α gutta percha cools down at a much more rapid rate than required. This routine cooling leads to contraction of gutta percha mass and thus would result in increased leakage at its interface with the canal walls. Other factors reported in literature that could be a contributing factor to increased leakage include decreased familiarity of operator with the thermoplasticised nonexistence systems, of guidelines to monitor progression of root filling material, presence of inadequate and poorly condensed gutta percha in apical one third of canal.27 Although in the present study, incremental obturation was carried out when using Obtura-II system, along with vertical compaction between each increment to minimize the voids that may form due to shrinkage on cooling, the quality of root filling was not evaluated by radiographic assessment and therefore it may be one of the reasons of increased leakage

values in this study group. Other studies have also reported a higher mean microleakage with the thermoplasticised obturation techniques. 7,26

Cold lateral compaction has been the gold standard obturation technique for decades because of the good clinical results that have been associated with this obturation technique.²⁸ In the present study, cold lateral condensation showed better sealing than thermoplasticised gutta percha. This can be credited to better length control of obturation material as indicated by the 'tug back' felt when gutta percha point is put to length.²²

In the present study, the degree of dye penetration was measured in millimetres; giving an objective assessment. Standardized measurements were captured for each specimen by using a calibrated camera attached to the microscope. 15% samples from each study group were randomly selected for measurement of dye penetration by a second assessor independent of the first set of readings. The mean correlation coefficient came out to be 0.81, indicating a good correlation between measurements carried out by the two assessors.

On the contrary, limitations of the present study include lack of radiographs post obturation in order to assess quality of obturation before sectioning of specimen for assessment. Also, dye penetration was the only method used for assessment of the sealing ability of obturation materials placed by different techniques.

CONCLUSIONS

Obtura-II along with Sealapex showed the maximum microleakage; indicated by the greatest dye penetration in this group. Cold lateral condensation with Sealapex had microleakage values that were less than the other group.

Recommendations:

Keeping in view the results of the study, for obturation of single rooted teeth the authors recommend cold lateral condensation with Sealapex sealer as it showed better sealability. Obtura-II and Sealapex should be used with caution in single rooted teeth as this group showed the maximum leakage. These results cannot be extrapolated to multi rooted teeth. Thus, a study should be conducted to assess the leakage in multi rooted teeth when obturated with different obturation techniques.

Acknowledgements:

The authors would like to acknowledge Dr. Syeda Mahvish Hussain, Dr. Sana Ehsan, Ms. Rizwana Naseem, Ms. Sadia Habib, Mr. Imran Lalani, Ms. Naila Badruddin and Mr. Muhammad Amin, who helped us out at various steps of conducting this study.

AUTHORS' CONTRIBUTION

MML: Literature Review, data collection, Conceptualization of study design, write-up, data entry, interpretation. FRK: Conceptualization of study design, data analysis, interpretation, proof reading.

REFERENCES

- Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965;20:340–9.
- Molander A, Reit C, Dahlen G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J 1998;31(1):1–7.
- Farea M, Masudi S, Wan Bakar WZ. Apical microleakage evaluation of system B compared with cold lateral technique: In vitro study. Aust Endod J 2010;36(2):48–53.
- WT Johnson JK. Obturation of the cleaned and shaped root canal system. In: HM Hargreaves SC, editor. Cohen's Pathways of the Pulp. St. Louis: Mosby; 2011. p.349–88.
- Leonard JE, Gutmann JL, Guo IY. Apical and coronal seal of roots obturated with a dentine bonding agent and resin. Int Endod J 1996;29(2):76–83.
- Inan U, Aydemir H, Tasdemir T. Leakage evaluation of three different root canal obturation techniques using electrochemical evaluation and dye penetration evaluation methods. Aust Endod J 2007;33(1):18–22.
- Rahimi S, Oskoee SS, Shahi S, Maljaei E, Abdolrahimi M, Mokhtari H, et al. In vitro comparison of apical microleakage following canal obturation with lateral and thermoplasticized gutta-percha compaction techniques. Afr J Biotechnol 2010;9(48):8235–40.
- Dultra F, Barroso JM, Carrasco LD, Capelli A, Guerisoli DMZ, Pécora JD. Evaluation of apical microleakage of teeth sealed with four different root canal sealers. J Appl Oral Sci 2006;14(5):341–5.
- Carrotte P. Endodontics: part 8. Filling the root canal system. Br Dent J 2004;197(11):667–72.
- Almeida JF, Gomes BP, Ferraz CC, Souza-Filho FJ, Zaia AA. Filling of artificial lateral canals and microleakage and flow of five endodontic sealers. Int Endod J 2007;40(9):692–9.
- WU MK, De Gee AJ, Wesselink PR, Moorer WR. Fluid transport and bacterial penetration along root canal fillings. Int Endod J 1993;26(4):203–8.
- Limkangwalmongkol S, Burtscher P, Abbott PV, Sandler AB, Bishop BM. A comparative study of the apical leakage of four root canal sealers and laterally condensed guttapercha. J Endod 1991;17(10):495–9.
- Punia SK, Nadig P, Punia V. An in vitro assessment of apical microleakage in root canals obturated with gutta-flow, resilon, thermafil and lateral condensation: A stereomicroscopic study. J Conserv Dent 2011;14(2):173–7.
- Bodrumlu E, Tunga U. Apical leakage of Resilon obturation material. J Contemp Dent Pract 2006;7(4):45–52.
- Sadr S, Golmoradizadeh A, Raoof M, Tabanfar MJ. Microleakage of Single-Cone Gutta-Percha Obturation Technique in Combination with Different Types of Sealers. Iran Endod J 2015;10(3):199–203.
- Elias I, Guimarães GO, Caldeira CL, Gavini G, Cai S, Akisue E. Apical sealing ability comparison between GuttaFlow and AH Plus: in vitro bacterial and dye leakage. J Health Sci Inst 2010;28(1):77–9.

- Sevimay S, Kalayci A. Evaluation of apical sealing ability and adaptation to dentine of two resin-based sealers. J Oral Rehabil 2005;32(2):105–10.
- Souza EM, Pappen FG, Shemesh H, Bonanato-Estrela C, Bonetti-Filho I. Reliability of assessing dye penetration along root canal fillings using methylene blue. Aust Endod J 2009;35(3):158–63.
- Al-Dewani N, Hayes SJ, Dummer PMH. Comparison of laterally condensed and low-temperature thermoplasticized gutta-percha root fillings. J Endod 2000;26(12):733-8.
 Schirrmeister J, Kielbassa A. Apikale Dichtigkeit von
- Schirrmeister J, Kielbassa A. Apikale Dichtigkeit von Wurzelkanalsealern auf Calciumphosphat-Basis im Vergleich zu marktüblichen Füllpasten. Dtsch Zahnärztliche Z 2005;60(8):446–56.
- Chawla A, Kapur R, Singh P. Assessment of apical seal obtained by three obturating techniques using dye penetration method and macro lens photography: an in-vitro study. Endodontology 2012;24:29–35.
- Tomson R, Polycarpou N, Tomson P. Contemporary obturation of the root canal system. Br Dent J 2014;216(6):315–22.
- 23. Goodman A, Schilder H, Aldrich W. The thermomechanical properties of gutta-percha. II. The history and molecular

chemistry of gutta-percha. Oral Surg Oral Med Oral Pathol 1974;37(6):954-61.

- Guigand M, Glez D, Sibayan E, Cathelineau G, Vulcain JM. Comparative study of two canal obturation techniques by image analysis and EDS microanalysis. Br Dent J 2005;198(11):707–11.
- Schilder H, Goodman A, Aldrich W. The thermomechanical properties of gutta-percha. Part V. Volume changes in bulk gutta-percha as a function of temperature and its relationship to molecular phase transformation. Oral Surg Oral Med Oral Pathol 1985;59(3):285–96.
- Varun K, Harpreet S, Rajinder B, Samrity P. Qualitative and Quantitative Comparative Evaluation of Sealing Ability of Guttaflow, Thermoplasticized Gutta Percha and Lateral Compaction for Root Canal Obturation: A Cohort, Controlled, Ex-Vivo Study. Oral Health Dent Manag 2013;12(3):155–61.
- Gutmann JL, Rakusin H. Perspectives on root canal obturation with thermoplasticized injectable gutta-percha. Int Endod J 1987;20(6):261–70.
- Peng L, Ye L, Tan H, Zhou X. Outcome of root canal obturation by warm gutta-percha versus cold lateral condensation: a meta-analysis. J Endod 2007;33(2):106–9.

Accepted: 23 July, 2017

Received:23 August, 2016 Address for Correspondence:

Dr. Maham Muneeb Lone, Assistant Professor, Department of Operative Dentistry, Jinnah Sindh Medical University, Rafiqui H.J Shaheed Road, Karachi-Pakistan **Email:** maham.mlone@gmail.com

Revised: 16 June, 2017