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Information to Authors

Chattagram International Dental College (CIDC) started its historical and memorable journey in the 2003 year. CIDC is the only Private Dental College in Chattogram which is smoothly running under the guidance of Chattogram University.

CIDC is approved by the Government of the Peoples Republic of Bangladesh and is recognised by the Bangladesh Medical and Dental Council (BMDC). CIDC is representing pioneer and exemplary academic and clinical oriented research institute of Bangladesh. About 65 Dental students completed their graduation from CIDC per annum.

Chattagram International Dental College commenced to publish a peer reviewed Journal from 1st January 2018 which is recognized by BMDC and having International Standard Serial Number (ISSN) 2707-2185. The journal intend to publish article of authors from any part of the globe, but has a special interest in publishing research articles of authors from Bangladesh and of relevance to developing countries. It interested in Editorial, Original (Research) articles, Special articles, Review articles, Short Communications, Case report and letters on new findings of Medical Science.

Chattagram International Dental College Journal is published in english, biannually eg. January and July with prior approval of Editorial board.

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- All tables should be numbered using Roman numerals (I, II).
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Unmounted glossy print, B-2 size with good contrast (600 pixels). 3 Images / Photographys / Legends are allowed for whole text.

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The article should accompany a declaration signed by author and co-authors which includes a statement that neither the article nor any part of its essential subtance table or figures is published in any journal nor submitted elsewhere for consideration of publication before appearing in this journal. The declaration form must be collected from the office of Editor-In-Chief

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Editorial

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Recent Challenges in Classification of Root Canal System

Shahiqul Jabbar[1*]

Root canal system is not a single canal running uniformly from canal orifice to apex; in fact it is very complex due to splitting and union of canals during its course to apex. Ideal requirements of a classification for root canal configuration is that it must define the tooth in terms of number of root, number of canal in each root and the course of canal from orifice to apex. At the same time it must be simple, accurate and clinically reliable[1,2].

Clinical classification of root canal system was given first by Weine FS et al. It was further elaborated by Vertucci FJ in 1984. Depending upon the configuration, root canal may exit apically through one or more apical foramen. Advancement in three dimensional imaging like cone beam computed tomography, micro-computed tomography as well as use of magnification have increased the number of reports on complex root canal anatomy. Many root canal configurations cannot be classified by Vertucci classification. Hence, various researchers also proposed newer classification[3-5].

Various morphological features on which root canal configurations are classified:

1. On the basis of number of canals from orifice to apex
2. On the basis of number of roots and number of canals in each root
3. On the basis of number of isthmus.

I. On the Basis of Number of Canals from Orifice to Apex

i) Weine FS et al was the first to categorize root canal configurations within a single root into four basic types as[3]:

- Type I (1-1): Single canal runs from orifice to apex
- Type II (2-1): Two canals arise from pulp chamber and during its course unite into one
- Type III (2-2): Two canals run separately from orifice to apex
- Type IV (1-2): One canal arises from floor of pulp chamber and during its course divides into two.

ii) Vertucci FJ elaborated Weine's classification[4]. He classified root canal systems into eight types as:

- Type I (1-1): Single canal runs from orifice to apex
- Type II (2-1): Two canals arise from pulp chamber which unite in its course into one
- Type III (1-2-1): One canal arises from pulp chamber and during its course splits into two. These two canals again unite into one before exiting from apex
- Type IV (2-2): Two canals run separately from orifice to apex
- Type V (1-2): One canal arises from floor of pulp chamber and during its course divides into two
- Type VI (2-1-2): Two canals start from pulp chamber, during its course; they unite into one and then again divide into two before exiting from root apex
- Type VII (1-2-1-2): One canal leave the pulp chamber which divide and again unite into in its course and finally divide into two before exiting from apex
- Type VIII (3-3): Three canals leave the pulp chamber and run independently towards the apex.

iii) Gulabivala K et al examined mandibular molars in a Burmese population and added seven additional configurations to Vertucci's classification as[5] : Type I to Type VII. These configurations classified 4 or 5 canals extending from the orifice also.

2. On the Basis of Number of Roots and Number of Canals in Each Root:

i) Zhang R et al gave classification for mandibular molars only according to the number of roots and the number of canals in each root[6].

3. On the Basis of Number of Isthmus:

Kim SY Divided Apex into 5 Types[7]:

- Type I: Two canals at root tip without any connection.

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- Type II: Two canals with incomplete connection between them.
- Type III: Three canals at root tip without any connection.
- Type IV: Extension of main canal resulting in a shape of tear.
- Type V: Two canals with complete connection between them.

Limitations

- Classification proposed by Weine FS et al Vertucci FJ et al were the most commonly used classification by various researchers in their studies[3,4]. Apart from eight types of root canal configurations suggested by additional types were reported in various studies[8].
- Advanced 3D imaging technology has revealed that the morphological characteristics of root canal system are highly complex[9].
- Another limitation of these classifications is that it did not explain in which root of multi-rooted teeth canals are encased[10].
- Division or union of canals is taking place in which part of root canal. Division of canal in the coronal, middle or apical third will be coded same e.g 1-2-1 can be coded for all canals in which bifurcation is taking place at coronal third, middle third or apical third. In Weine FS Type II/Vertucci FJ Type II (2-1) Vertucci FJ Type III (1-2-1) Type XXII (3-2-1) two canals are uniting into one, but in which part of root canal space two canals are uniting is not clear[3,4,11].
- These classifications do not consider the position of apical foramen through which root canal exit[12].
- The assessment of apical canal configurations may vary e.g some apical bifurcations could either be classified as an apical delta/ramification or a division from the main canal (Type 1-2)[13].

7. It is difficult to memorize all classifications and hence Vertucci FJ classification and additional configuration given by Gulabivala K are the main classification used for communication purposes[4,5,14].

● Four Digit Formula According to Course of Canal in Coronal, Middle and Apical Third:

Briseno-Marroquin B et al divided root canal into thirds and gave a digit to each third[15]. Fourth digit was given to number of foramina. But this classification was not clinically applicable, as it is not possible to divide root canal clinically into thirds on intraoral radiographs. Secondly number of foramen cannot be evaluated accurately by intraoral periapical radiographs.

● Morphological Features to be Considered While Giving a Nomenclature System:

i) Tooth type

ii) Root in which configuration is encased

iii) Root canal configuration from orifice to apex

iv) Location at which root canal exit from root apex

v) Anatomic variations like radix molar, C shaped canal etc.

● New Proposal:

i) Tooth number: The tooth number can be written using FDI system of nomenclature

ii) Nomenclature of root written on the right side of the tooth number

iii) The course of the canal in each root mentioned in round brackets written on right side of the nomenclature of each root

iv) Number of foramen through which canal is exiting at the apex and mentioned after putting slash to course of canal.

v) Proposal for anatomic variations:

- C- Shape canal can be mentioned by letter “C” added to the left of the tooth number. The fusion between canals can be depicted (-) between canals
- Taurodontism can be mentioned by adding letter “T” added to the left of the tooth number.
- Single root can be mentioned by adding letter “R” added to the right of the tooth number.
- Individual names like Radix paramolaris, Radix entomolaris, middle mesial canal etc are not required. Additional root of Radix paramolaris can be written Mesiobuccal (MB) or Distobuccal (DB) and similarly additional root of Radix entomolaris as Mesiolingual (ML) or Distolingual (DL) depending on its anatomical position. Similarly middle mesial canal i.e., canal in between MB and ML canal can be written as 3-3, 3-2 or 3-1 depending on the course of middle canal from orifice to apex.
- For example: 47M(1-2-1-2/2)D(1-1-1/1) can be represented as right mandibular second molar with mesial root having configuration –one canal arise from pulp chamber, during its course divides into two and then again unite into one and then divide into two which exits through two foramen. In the distal root one canal continues from orifice to apex and exit through one foramen.

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Conclusion

All classification reported, have their advantages and limitations. The proposed nomenclature system takes into account the tooth type, number of roots and course of canal in each root. This classification is a simple, reliable, accurate and easy for communication as it is guided by course of canal. Further the proposed system can define any new configuration. Hence it can be used both for clinical and research work.

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Original Article

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Changes of Soft Tissue Facial Profile of Patients with Class 1 Malocclusion by Non-extraction Orthodontic Treatment

Ripan Das[1*] Gazi Shamim Hasan[2] Tanzila Rafique[3] Mohammad Azizur Rahman[4]
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Abstract

Background: *Non extraction method of doing orthodontic treatment may result different soft tissue changes in view of facial profile. Orthodontic treatment through non-extraction may contribute different results in treating class-I malocclusion cases. To evaluate effect of non-extraction orthodontic treatment on the facial profile of patient with class I malocclusion.* ***Materials and methods:*** *35 patients of non-extraction orthodontic treatment were collected by consecutive sampling from orthodontic department of Bangabandhu Sheikh Mujib Medical University from November 2017 to April 2018. Facial profile of these patients evaluated by pre-treatment and post-treatment facial photographs, cephalometric tracings. The photographs were 1 4 life size in all three views: profile, frontal, and smile. The quantitative measurements selected were i) an angular measurement—the Z-angle of Merrifield and ii) a linear measurement—the lower lip to the esthetic line of Ricketts, called in this study, the "E-value." The Z-angle and E-value are chosen for their ease and accuracy to measure and their relevance to lip position and lip change, thus making comparisons relevant and easy to evaluate.* ***Results:*** *In this study, no significance difference was found in treating class-I malocclusion patients through non-extraction method.* ***Conclusion:*** *From the study results, it is concluded that non-extraction treatment has no significant effect on soft tissue profile for treating the class-I malocclusion orthodontic patients.*

Key words

Orthodontic treatment; Soft tissue profile; Non-extraction method.

Introduction

Orthodontics is the branch of Dentistry which mainly deals with malocclusion and dentofacial deformities and their correction for optimal function and esthetics. Orthodontic treatment should not focus only on occlusal relations, but also on facial esthetics, in particular profile esthetics[1]. In the present era, several treatment modalities emphasize soft tissue paradigm[2,3]. Wuerpel discussed the changes in soft tissue that must be considered during orthodontic treatment, instead of moving teeth without anticipating soft tissue outcomes after treatment[4].

The aesthetic impact on soft tissues is a deciding factor for extractions in orthodontics[5]. The parameters which differentiate between extraction and non-extraction treatment modalities in Class I border line cases are upper and lower lip protrusion in relation to E-plane and the Sn-Pg' line, lower lip protrusion in relation to the True Vertical Line (TVL) upper lip thickness, nasolabial angle and interlabial gap. These parameters can be used as guidelines in decision making to choose either extraction or nonextraction in Class I borderline cases[6].

Merrifield's study of facial profiles in a sample of 120 untreated normal patients and treated patients with pleasing facial esthetics led to the development of the "Z-angle" to quantify balance, or lack thereof, of the lower facial profile[7]. The Z-angle is the inner-inferior angle formed by the intersection of the Frankfort horizontal plane and the "profile line" (A line that is tangent to the soft tissue chin and the most prominent lip). He found the normal Z-angle range in his sample to be 72° to 83°.

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To evaluate effect of non-extraction orthodontic treatment on the facial profile of patient with class I malocclusion.

i) To measure Z angle and E value at the border line before non-extraction.

ii) To measure Z angle and E value after completing treatment.

Materials and methods

35 patients of non-extraction orthodontic treatment were collected by consecutive sampling from Orthodontic Department of Bangabandhu Sheikh Mujib Medical University From November 2017 to April 2018. Facial profile of these patients evaluated by pretreatment and post-treatment facial photographs, cephalometric tracings. The photographs were 1 4 life size in all three views: profile, frontal, and smile. The quantitative measurement selected was i) an angular measurement-the Z-angle of Merrifield and ii) a linear measurement-the lower lip to the esthetic line of Ricketts, called in this study, the "E-value." The Z-angle and E-value are chosen for their ease and accuracy to measure and their relevance to lip position and lip change, thus making comparisons relevant and easy to evaluate. As described earlier, the Z-angle is the angular measurement made by the profile line and the Frankfort horizontal plane. The E-value is the distance in millimeters from the most anterior point of the vermillion border of the lower lip to the esthetic line. If the lower lip is in front of the esthetic line, the E-value is positive. If the lower lip is behind the esthetic line, the E-value is negative. The more protrusive the lip profile was, the less the Z-angle was. The more retrusive the lip profile was, the greater the Z-angle was. In general, as the lip profile position became more protrusive in its relationship to the chin, the Z-angle decreased and the E-value became more positive. As the lip profile position became more retrusive in its relationship to the chin, the Z-angle increased and the E-value became more negative. In this study, Paired sample t-test was done to compare the pretreatment and post-treatment measurements of Z-angle and E-value.

The purpose of the study was to quantitatively and visually compare the facial profiles and soft tissue balance found in patients before starting the treatment and after finishing the treatment through non-extraction orthodontic treatment. A sample of 35 for each group was collected over a 12 months period. By consecutive sampling, thirty-five of these patients were treated after non-extraction. The patient records were used in the study consisted of pre-treatment and post-treatment facial photographs, cephalographs, and tracings. The photograph was 1 4 life size in all three views: profile, frontal, and smile. The quantitative measurement selected was i) an angular measurement—the Z-angle of Merrifield and ii) a linear measurement-the lower lip to the esthetic line of Ricketts, called in this study, the "E-value." The Z-angle and E-value are chosen for their ease and accuracy to measure and their relevance to lip position and lip change, thus making comparisons relevant and easy to evaluate. As described earlier, the Z-angle is the angular measurement made by the profile line and the Frankfort horizontal plane. The E-value is the distance in millimeters from the most anterior point of the vermillion border of the lower lip to the esthetic line. If the lower lip is in front of the esthetic line, the E-value is positive. If the lower lip is behind the esthetic line, the E-value is negative.

The more protrusive the lip profile was, the less the Z-angle was. The more retrusive the lip profile was, the greater the Z-angle was. In general, as the lip profile position became more protrusive in its relationship to the chin, the Z-angle decreased and the E-value became more positive. As the lip profile position became more retrusive in its relationship to the chin, the Z-angle increased and the E-value became more negative. In this study, pretreatment and post-treatment measurements were compared within the non-extraction group. Total 35 for each group patients included non-extracted patients.

Results

31.4% of patients were in the age of 15-17 years. 28.6%, 14.2%, 17.2%, 8.6% were distributed in the age of 13-15 years, 17-19 years, 19-21 years and 21-23 years respectively. Mean and standard deviation of age was 17.21±1.38 year. Treatment period was 2.04±0.41 year (Table I).

Table I : Pretreatment and Post-treatment Mean Values and Standard Deviations of Z-angle and E-value.

Treatment status	E-value (Mean±sd) in millimeter (mm)	
Pre treatment	73.82±1.9	-1.72±1.2
Post treatment	75.35±2.17	-2.66±0.99

In Table II the Z-angle and E-value were expressed as mean±sd and showing that in non-extraction cases Z-angle change from 73.82±1.9 to 75.35±2.17 and E-value from -1.72±1.2 to -2.66±0.99.

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Table II : Comparison of pretreatment and post-treatment measurements (Z-angle and E-value) in class-I.

Parameters	Pretreatment		Post treatment		Difference between pre and post treatment		p value
	Mean	SD	Mean	SD	Mean	SD	
Z-angle in degree (^{0})	73.82	1.9	75.35	2.17	1.53	0.27	0.00001
E-value in millimeter (mm)	-1.72	1.2	-2.66	0.99	-0.94	8.2	0.9996

Note: Ignore negative sign in case of differences.

Statistically significant p value 0.00001 (<0.05) was found in comparison of Z-angle at the level of 95% confidence level (At p<0.05). But E-value did not show any significant difference.

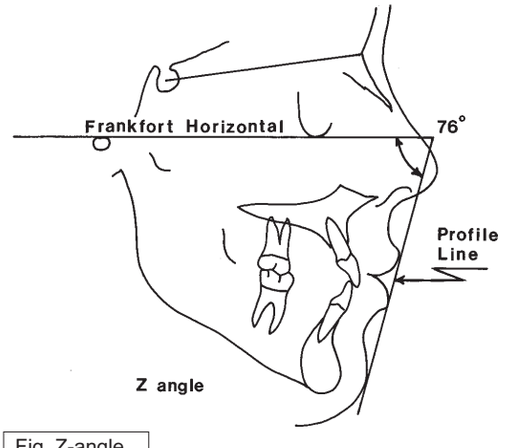

Fig. Z-angle measurement

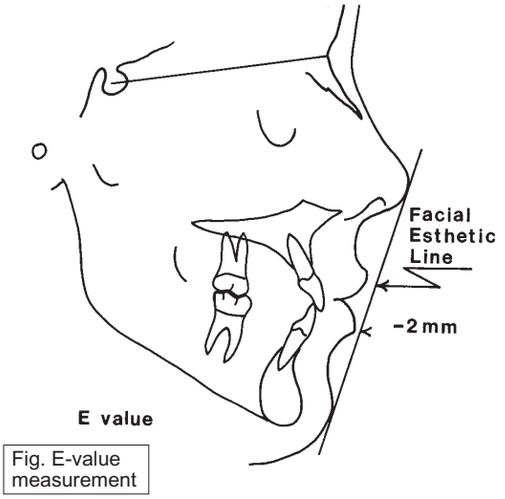

Fig. E-value measurement

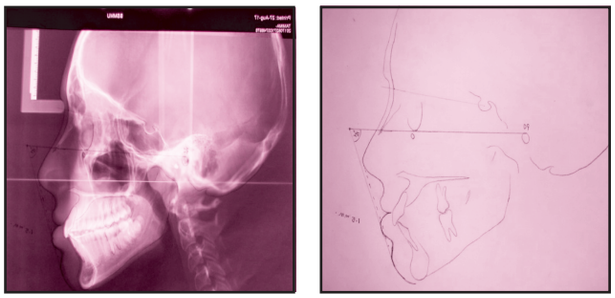

Figure 1 : Pretreatment Cephalometric tracing (Non-extraction case).

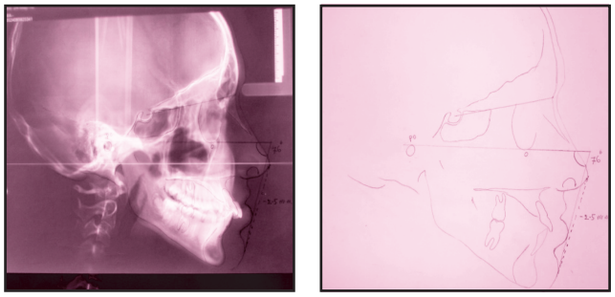

Figure 2 : Posttreatment Cephalometric tracing (Non-extraction case).

Discussion

According to Ricketts, a distance of -3.0 m.m. to ±2.0 m.m. from the lower lip to the E line should be considered normal in 15 years old[8]. The pretreatment E-value for the non-extraction group was -1.72 mm. The post treatment E-values of the non-extraction group was -2.66 mm. These all values were within normal range but these differences show no significance in pre and post treatment non-extraction groups.

Considering the results of this study, the soft tissue profile was important guideline for successful orthodontic treatment though non-extraction modalities are still controversial. It is noted that the assessment of facial profile should be a continuous learning process for orthodontists, since patients are increasingly concerned about the effect that orthodontic treatment can induce in facial esthetics.

Conclusion

With the limitation of this study, it can be concluded that-

i) The soft tissue facial pro les of patients with Class-I malocclusion can be treated with non-extraction treatments.

ii) No significant differences were seen in soft tissue changes of orthodontic patients treated with non-extraction.

iii) Non-extraction group facial profile value (Z-angle and E-value) are averages within the normal range. [Ideal range of Z-angle: 70^0 - 80^0 and e-value: -2±3mm]

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Disclosure

All the authors declared no competing interest.

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Original Article

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Evaluation of Impact on Antibiotic Prescribing in A University Hospital Before and After Intervention and Survey on Completion of Duration of Antibiotic Therapy

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Abstract

Background: *Antibiotic resistance is a global problem with a strong impact on morbidity and mortality. This resistance found to have link with the using pattern of antibiotics. Not completion the duration of prescribing antibiotic by the patient is considered irrational use for this drug. This study was to see the doctor's prescribing pattern and patient's compliance about the completion of duration of antibiotic therapy.* ***Materials and methods:*** *This was a 'Formative Research' conducted as a before-after in Bangabandhu Sheikh Mujib Medical University (BSMMU) from July 2015 to July 2018. For doctor's prescribing pattern was evaluated by prescription audit and patient awareness was observed by compliance. Educational intervention was implicated and its Impact was evaluated by measuring change in proportion of prescribed antibiotics before and after intervention.* ***Results:*** *Overall 68.0% of the admitted and 61.5% of outdoor patients received Antibiotic in BSMMU Hospital. After intervention, proportion of patient received antibiotic significantly reduced. Among the prescription of four departments 100% antibiotics were prescribed with duration in indoor and outdoor but 51% patient's adherence were observed about the completion of duration of antibiotic therapy.* ***Conclusion:*** *Implementation of antibiotic guideline, awareness and managerial intervention to improve antibiotic prescribing and completion of total duration of prescribed antibiotic therapy will play a vital role in containment of antibiotic resistance.*

Key words

Antibiotic resistance; Antibiotic Guideline; Antibiotic Stewardship Program (ASP).

Introduction

Bangladesh is going through a transition in health, however the major causes of morbidity and mortality are still infective disease and therefore antibiotics are the most widely used medicine[1,2]. Inappropriate use of Antibiotics led to emergence of resistance that finally results into loss of their effectiveness[3,4]. Studies conducted in Bangladesh during last decades revealed that prescribing of antibiotics are generally irrational[5,6]. Moreover, consumption of antibiotics with short time than the prescribing time perhaps worsened the situation[7]. Few educational and managerial interventions were found successful in Bangladesh[5,7,8].

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Antibiotic Stewardship Program (ASP) is considered as the most effective approach to improve antibiotic prescribing[9-11]. ASP encourage clinician to improve quality of care through better infection cure rate[12,13]. On this background, intervention was designed in this study to support the implementation of rational Antibiotic prescribing. The experience and evaluation of the patient's compliance would provide important information about the knowledge, attitude and perception of patients towards the completion of antibiotic therapy.

Materials and methods

The 'Formative Research' was conducted as a before-after in Bangabandhu Sheikh Mujib Medical University (BSMMU) from July 2015 to July 2018 and the impact was evaluated in the selected department e.g, Department of Internal Medicine, Surgery, Obstetrics & Gynecology and Pediatrics. Before initiating the actual study, ethical clearance was obtained from the Institutional Review Board of BSMMU.

For this study, 100 antibiotic containing prescriptions from each department, total 400 were analyzed. This 100 included 50 indoor and 50 outdoor prescriptions in which antibiotics were prescribed by the doctor. A data collection form was used to collect the information from the prescription. Data collection were performed just before and after of giving intervention. In the prescription it was observed that weather doctor mentioned the duration of antibiotic therapy or not

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and antibiotic drug completion date for individual patient was collected. In addition, a face to face interview was done to the patient; to asses that they were well informed about the importance of completion of Antibiotic therapy by the doctor. Their contact number was also collected through this interview which was used to further telephone interview. At the particular date when the individual antibiotic therapy date mentioned in the prescription was completed this telephone interview was performed to know the patient's compliance regarding completion of duration. If he or she not completed the full duration, the reason behind it was evaluated through this interview.

Description of the Intervention

Antibiotic Guideline: An online Antibiotic Guideline was launched on 14th December 2015 in the presence of administration of the University. Stewardship was executed by the active participation of the key prescribers during the process of development of guideline as well as the formal commitment, endorsement and persuasion from of the top level management of the University.

Reminders: Repeated reminders were sent to the prescribers and stakeholders through SMS.

Academic Detailing: Antibiotic awareness week 2016, 2017 was celebrated in the University which added a great value to create awareness among the prescriber. Face-to-face educational visit conducted by the provision of scientific evidences to the prescribers.

Post-intervention Data Collection

Post interventional data was collected on January 2018 as same as pre interventional data collection procedure to evaluate the impact of intervention.

Statistical Analysis

Data was compiled, presented and appropriate statistical test (Paired proportion test and unpaired t-test) was applied to draw the expected conclusion. Microsoft Excel 2007 was used for the statistical analysis. p value was calculated by test statistic (t value) using online calculator against corresponding degree of freedom (df).

Results

68.0% of the admitted and 61.5% of outdoor patients received antibiotic in BSMMU Hospital. Table I revealed that in the Department of Obstetrics & Gynecology 78.0% followed by the Departments of Surgery 74.0%, Internal Medicine 62.0% and Pediatrics 58.0%. In outpatients the highest 68.0% antibiotics were prescribed in the Department of Internal Medicine, Department of Obstetrics & Gynecology 64.0%, Surgery 60.0% and Pediatrics 54.0%.

Table I: Proportion of patients of four departments of BSMMU Hospital received antibiotics.

	Dept. of Internal Medicine	Dept. of Surgery	Dept. of Obstetrics & Gynecology	Dept. of Pediatrics
Inpatients	(n= 50)	(n=50)	(n=50)	(n=50)
	62.0%	74.0%	78.0%	58.0%
	(31/50)	(37/50)	(39/50)	(29/50)
Outpatients	(n= 50)	(n=50)	(n=50)	(n=50)
	68.0%	60.0%	64.0%	54.0%
	(34/50)	(30/50)	(32/50)	(27/30)

n= Total number of prescriptions reviewed
Percentage = Total number of prescriptions contained antibiotic/Total number of prescriptions reviewed to get those prescriptions.

After intervention, proportion of patient received antibiotic significantly reduced. Among the patients admitted in the Departments of Internal Medicine, proportion of patient received antibiotic reduced from 62.0% to 37.0% and Department of surgery 74.0% to 49.0% respectively, both of which were statistically significant ($p < 0.01$). In the Departments of Obstetrics & Gynecology, no statistically significant change was observed (Table II). Lastly in the department of Pediatrics proportion reduced 58.0% to 40.0% which was statistically significant.

Table II: Comparison between before and after intervention in proportion of antibiotic use in four indoor departments of BSMMU Hospital.

	Proportion of Patients Received Antibiotic		
	Before Intervention (n=63)	After Intervention (n=135)	p value
Dept. of Internal Medicine	62.0%	37.0%	0.01
Dept. of Surgery	74.0%	49.0%	0.01
Dept. of Obstetrics & Gynecology	78.0%	73.0%	0.50
Dept. of Pediatrics	58.0%	40.0%	0.01

Paired proportion test was done, $p \leq 0.05$ was considered as statistically significant.

Table III shows, comparison between before and after intervention in proportion of patient received antibiotic in outdoor. In the four departments reduction proportion of patient received antibiotic reduced significantly ($p < 0.01$).

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Table III : Comparison between before and after intervention in proportion of antibiotic use in four outdoor departments of BSMMU Hospital.

	Proportion of patients received antibiotic		
	Before Intervention (n=63)	After Intervention (n=135)	p value
Dept. of Internal Medicine	68.0%	35.0%	0.01
Dept. of Surgery	60.0%	31.0%	0.01
Dept. of Obstetrics & Gynecology	64.0% (30/33)	33.0% (30/35)	0.01
Dept. of Pediatrics	54.0%	40.0%	0.01

Paired proportion test was done, $p \leq 0.05$ was considered as statistically significant.

Among the prescription of four departments 100% antibiotics were prescribed with duration in indoor and outdoor. This scenario was common in case of before and after intervention. Table IV illustrates this scenario.

Table IV : Percentage of prescription containing antibiotics with duration in the inpatients and outpatients of BSMMU Hospital before and after intervention.

	Dept. of Internal Medicine	Dept. of Surgery	Dept. of & Obstetrics & Gynecology	Dept. of Pediatrics
Inpatient	100%	100%	100%	100%
Outpatient	100%	100%	100%	100%

The result of telephone tracking of the patients was not satisfactory. Among the 400 patients 17 patients were not responding to their contact number. Rest of the patients those were tracked by the phone, 186 (48.5%) of them was failure to continue the Antibiotic up to the date instructed in the prescription which was statistically significant. Among the 186 patients, 123 were stopped to taking the drug for felt better. 34 patients were not get any symptomatic relief so they stopped taking drug. Rest 29 patients had no significant reason to failure to continue the drug. For the understanding of the reason behind incompliance, further investigation was performed through the telephone conversation. Different issues were pointed out and measures for successful implementation were advised (Table V).

Table V : Knowledge, attitude and perception of the patients about completion of duration of antibiotic.

Knowledge, Attitude and Perception
i) Almost half of the patients (210 patients) did not know that which is antibiotic among the prescribed drug.
ii) More than half of the patients (318) had not any perception about the importance of completion Antibiotic course.
iii) None of them were counseled or instructed verbally to complete the duration of Antibiotic by the prescriber or dispenser.
iv) Among the 186 patients who were failure to continue the Antibiotic up to the date instructed in the prescription, 123 were stopped to taking the drug for felt better.
v) 34 patients were not get any symptomatic relief by taking drug so they stopped.

Discussion

This finding corresponds with the result of studies conducted in the similar hospital[14,15]. The higher prescribing rate in the Departments of Surgery and Obstetrics & Gynecology might be prophylaxis or therapeutic purpose. Another study revealed almost same result like as 81.9% of patients admitted in the department of Obstetrics & Gynecology which was followed by Surgery 78.5%, Internal Medicine 47.0% and Pediatrics 46.7%[16]. The pattern of preference is similar to that of Akhloufi et al. (2015) study[17]. This pattern assumed more antibiotic used in Departments of and Obstetrics & Genealogy and Surgery to be prophylactic use before surgeries and the major causative microbes in abdominal surgeries are similar[18]. It may be noted that only 11.1% is contribution of medical prophylaxis[19]. On the other hand, shows antibiotic prescribing in outdoor is double which is vice versa of the findings of this study[20].

Antibiotic Guideline, reminder, academic detailing is considered as a common intervention to improve the situation[21]. Orientation and motivational programs were supportive measures to improve the situation. But adherence to the intervention is not up to the mark and the possible reasons of noncompliance are inadequate participation of the prescribers, communication gap, lack of regular activities[14, 22].

To evaluate the impact of intervention, data was collected after the implication of intervention. A significant reduction ($p<0.01$) in the proportion of antibiotic prescribing and used in department of Internal Medicine, Surgery and Pediatrics in admitted patients and

in all department in outdoor. This might be explained by the improved awareness of the prescriber about antibiotic usage. A meta-analysis mentioned about the scope of this type of reduction in antibiotic prescribing[23]. In this study, there was no significant change in antibiotic prescribing of admitted patients in the departments of Obstetrics & Gynecology probably due surgical cases.

The study attempted to observe the completion of duration of prescribed drug which is an essential part of rational use of antibiotic. From the prescriber end among the prescription of four departments 100% antibiotics were prescribed with duration in indoor and outdoor. This scenario was common in case of before and after intervention. But from the consumer end the result was not satisfactory. About 48.5% patients were failure to continue the antibiotic up to the date instructed in the prescription. Among the defaulter more than two third were stopped to taking the drug for better feeling and less than one third was did not get any symptomatic relief so they stopped taking drug. Rest 29 patients had no significant reason to failure to continue the drug. Probably this dimension of antibiotic use was never studied before so comparison with other studies was not visible. The present study attempted to establish a new platform, which is innovative one. For the understanding of the reason behind incompliance, telephone interview was performed and different issues were pointed out. Among the issues patient understands level about the importance of completion of antibiotic duration was mainly focus. Beside this symptomatic relief or persistence of symptom are the other reasons of noncompliance.

Conclusion

Considering the above findings, this study confirmed that proper intervention is mandatory to improve the current situation. Awareness, managerial intervention and some scientific issues can be considered as a model intervention to improve the prescriber end prescribing. Fill the gap of knowledge and perception of the user end is make a bridge for the rational use of antibiotic which is most effective way to combat antibiotic resistance. It's clear that a comprehensive approach can bring a positive dimension in antibiotic utilization.

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Disclosure

Both the authors declared no competing interest.

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Original Article

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Assessment of Glycated Hemoglobin (HbA1c) For Diagnosis of Type 2 Diabetes in Bangladeshi Adults

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Abstract

Background: *HbA_{1c} is commonly used to evaluate the long-term glycaemic control in diabetic patients. An HbA_{1c} cut point of 6.5% is also recommended by the World Health Organization (WHO) as an additional tool for diagnosing diabetes. However, the recommendation is conditional and the quality of evidence is not high showing significant geographical and racial variations of results and preferred cut-offs. So, this study aimed to assess the performance of HbA_{1c} for diagnosis of type 2 diabetes in Bangladeshi adults using Oral Glucose Tolerance Test (OGTT) as reference.* ***Materials and methods:*** *This diagnostic test evaluation study was conducted from January 2016 to December 2016 in the Outpatient Department (OPD) of Medicine and Department of Biochemistry of Chattogram Medical College Hospital. Subjects of 40 to 70 years without known history of diabetes had undergone OGTT. Of them, 100 individuals fulfilling the glucose-based WHO diagnostic criteria of diabetes were enrolled in Group A. 80 age-sex matched healthy subjects from the community with normal OGTT results were included in Group B. The subjects were recruited purposively.* ***Results:*** *Receiver Operating Characteristic (ROC) curve revealed that at the WHO recommended cut-off of 6.5%, the sensitivity and specificity of HbA_{1c} for diagnosing diabetes were 95% and 93.75% respectively along with excellent positive and negative predictive values. But the highest diagnostic sensitivity (100%) and specificity (93.75%) of HbA_{1c} were seen at the cut-off of 6.15%. Besidesthegood diagnostic performance, HbA_{1c} maintained strong correlations very similar to that of fasting plasma glucose with most of the components of metabolic syndrome in the newly diagnosed type 2 diabetic patients.* ***Conclusion:*** *In our study of healthy and newly diagnosed type 2 diabetic patients, HbA_{1c} demonstrated excellent sensitivity and specificity for diagnosing diabetes where OGTT was used as the gold standard.*

Key words

HbA_{1c}; Diagnosis; Type 2 diabetes; Glucose; Metabolic syndrome.

Introduction

Diabetes is a global health problem with rapidly increasing prevalence in developing as well as developed countries. Until recently, the diabetes mellitus was diagnosed mostly by Oral Glucose Tolerance Test (OGTT) which includes both fasting plasma glucose and2-hour plasma glucose tests. Glycated Haemoglobin (HbA_{1c}) on the other hand, is typically used to assess the glycaemic control in diabetic patients over the past 2-3 months.It offers the benefits of more convenient sampling, smaller day-to-day variability and greater pre-analytical stability than plasma glucose testing[1-2]. Therefore, in 2011, soon after the recommendation of American Diabetic Association (ADA) the World Health Organization (WHO) also endorsed HbA_{1c} as an additional test to diagnose diabetes providing that stringent quality assurance tests are in place, assays are standardised to criteria aligned to the international reference values, and there are no conditions present which preclude its accurate measurement[3-4]. However, HbA_{1c} isinfluenced by many other factorslike age and ethnicity[5-7]. Thus, using the current WHO-recommended cut-offof ≥6.5, although improvedspecificity, lacked sensitivity in many Asian studies[8-10]. So, in this study, we aimed to evaluate the overall performance of HbA_{1c} for diagnosis of type 2 diabetesand to determine the optimal HbA_{1c} cut-offsfor Bangladeshi adults using Oral Glucose Tolerance Test (OGTT) as reference.

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Materials and methods

This diagnostic test evaluation studywas conducted from January 2016 to December 2016 in the Outpatient Department (OPD) of Medicine and Department

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of Biochemistry of Chattogram Medical College Hospital after taking proper permission from the concerned departments and ethical review committee. Informed, written consent was taken from all the participants. Diabetic suspects of 40 to 70 years having no history of diabetes,were screened with Oral Glucose Tolerance Test (OGTT). Of them, 100 individuals fulfilling the WHO diagnostic criteria (Glucose-based) of diabetes were taken in Group A[11]. 80 age-sex matched healthy subjects from the community with normal OGTT results were included in Group B. Subjects were purposively recruited following the undermentioned inclusion and exclusion criteria.

Inclusion criteria for Group A: Persons aged from 40 – 70 years diagnosed diabetic for the first time in our tests with no other known pathology.

Exclusion criteria for Group A: Previously knowndiabetes, hemoglobinopathies, anaemia, splenomegaly, chronic renal or liver disease, carcinoma, pregnancy, on aspirin, antiretroviral agents, drugs that affect blood glucose, vitamin C and E supplementation, iron, vitamin B_{12} or folate administration or their deficiencies, history of splenectomy, recent blood transfusion, smoking, alcohol consumption.

Inclusion criteria for Group B: Healthy individuals from community aged 40-70 years.

Exclusion criteria for Group B: Subjects known or suspected to have any disease or pathology, pregnancy, history of splenectomy, recent blood transfusion, smoking, alcohol consumption, on drugs that may affect blood glucose or haemoglobin glycation.

Plasma glucose was determined by glucose oxidase method in Siemens Dimension clinical chemistry system. Plasma lipid profiling was also done on the same system. Fasting plasma insulin was estimated by direct chemiluminescent technology in ADVIA Centaur (Siemens) system. HbA_{1c} was measured using High-Performance Liquid Chromatography (HPLC) method in a Bio-Rad D10 system. Insulin resistance was calculated using Homeostatic Model Assessment Insulin Resistance (HOMA-IR), [HOMA-IR = fasting insulin (mIU/L) × fasting glucose (mmol/L)/ 22.5] higher values representing greater insulin resistance. Those with HOMA-IR value > 2.6 were categorized as insulin resistant. The reference value of fasting plasma insulin was up to 12 mIU/L[12]. Metabolic syndrome was defined as per revised criteria of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III). For elevated waist circumference or central obesity, cut-points of ≥90 cm in men and 80 cm in women were applied that appears to be appropriate for the Asians[13]. Statistical analyses were performed using Statistical Package for Social Science (SPSS) for Windows version 22.0. p values <0.05 were considered statistically significant. Quantitative data were expressed as mean ± SEM and qualitative data were expressed in frequency and percentage. Relevant statistical tests of significance were done as appropriate.

Results

Table I: Characteristics of Group A and Group B.

Traits	Group A (Diabetics) n = 100	Group B (Healthy subjects) n = 80	p value
Age (years)	48.50 ± 0.66	46.07 ± 0.79	>0.05
Male sex(%)	45.00	42.50	>0.05
BMI(kg/m^2)	25.16 ± 0.13	22.42 ± 0.16	<0.0001
FPG (mmol/L)	8.68 ± 0.15	5.27 ± 0.03	<0.0001
2-h glucose (mmol/L)	13.96 ± 0.25	6.99 ± 0.06	<0.0001
HbA_{1c}(%)	8.24 ± 0.15	5.58 ± 0.05	<0.0001

The above table shows that there were no significant differences in age and sex proportions between the two groups but BMI and glycaemic parameters were significantly different.

Table II: Evaluation of HbA_{1c} as a diagnostic test for type 2 diabetes taking glucose-based WHO criteria as gold standard (HbA_{1c} diagnostic cut-off used was • 6.5%) n = 180.

	Disease				
Test	**Present**	**n**	**Absent**	**n**	**Total**
Positive	True Positive	a= 95	False Positive	c= 5	a + c = **100**
Negative	False Negative	b= 5	True Negative	d= 75	b + d = **80**
Total		a + b = **100**		c + d = **80**	

Statistic	Formula	Value	95% CI
Sensitivity	$\frac{a}{a+b}$	95.00%	88.72% to 98.36%
Specificity	$\frac{d}{c+d}$	93.75 %	86.01% to 97.94%
Positive Likelihood Ratio	$\frac{Sensitivity}{1-Specificity}$	15.20	6.50 to 35.56
Negative Likelihood Ratio	$\frac{1-Sensitivity}{Specificity}$	0.05	0.02 to 0.13
Disease prevalence	$\frac{a+b}{a+b+c+d}$	55.56%	47.98% to 62.95%
Positive Predictive Value	$\frac{a}{a+c}$	95.00%	89.04% to 97.80%
Negative Predictive Value	$\frac{d}{b+d}$	93.75 %	86.43% to 97.25%
Accuracy	$\frac{a+d}{a+b+c+d}$	94.44%	90.02% to 97.30%

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HbA_{1c} ≥6.5% cut-off showed high sensitivity and specificity for diagnosing type 2 diabetes with very good positive and negative predictive values.

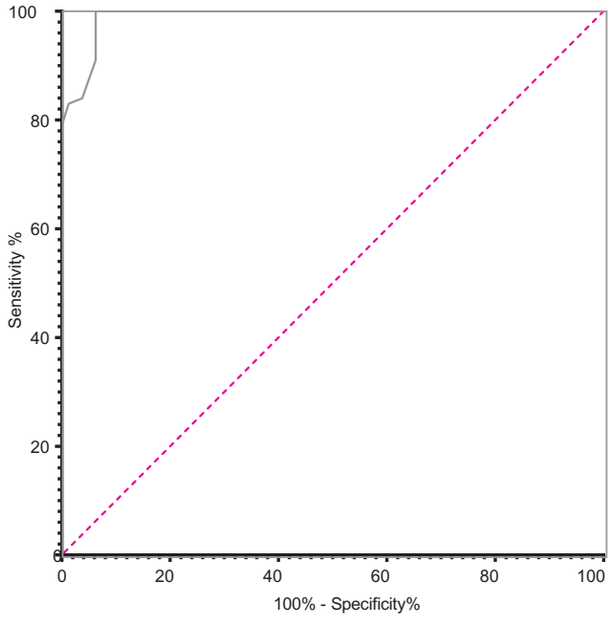

Figure 1: Receiver Operating Characteristic (ROC) curve to compare the sensitivity and specificity for all possible HbA_{1c} cut-offs.Area = 0.9904, Std. Error = 0.004842, 95% confidence interval = 0.9809 to 0.9999, p value <0.0001, n = 180.

Table III: Sensitivity and specificity for different HbA_{1c} cut-offs to diagnose type 2 diabetes, n = 180.

HbA_{1c} cut-off	Sensitivity%	95% CI	Specificity%	95% CI
> 6.15	100.0	96.30% to 100.0%	93.75	86.19% to 97.30%
> 6.25	99.00	94.55% to 99.95%	93.75	86.19% to 97.30%
> 6.35	97.00	91.55% to 99.18%	93.75	86.19% to 97.30%
> 6.50	95.00	88.82% to 97.85%	93.75	86.19% to 97.30%
> 6.65	91.00	83.77% to 95.19%	93.75	86.19% to 97.30%

Highest diagnostic sensitivity and specificity of HbA1c were seen at > 6.15% cut-off, but WHO recommended cut-off (≥ 6.5%) also had excellent sensitivity and specificity.

Table IV: Correlations of fasting plasma glucose and $HbA1_c$ with different components of metabolic syndrome in diabetic subjects, n = 100

Correlation coefficient (r) with	Fasting glucose	HbA_{1c}	z	p
BMI	+0.33	+0.44	-0.90	0.37
Waist	+0.50 (n=45, male)	+0.54 (n=45, male)	-0.25	0.80
circumference	+0.41(n=55, female)	+0.63 (n=55, female)	-1.56	0.12
Systolic BP	+0.49	+0.48	+0.09	0.93
Diastolic BP	+0.40	+0.44	-0.34	0.73
Insulin	+0.28	+0.26	+0.15	0.88
HOMA-IR	+0.70	+0.57	+1.53	0.13
Triglyceride	+0.48	+0.49	-0.09	0.93
HDL-C	-0.42 (n=45, male)	-0.65 (n=45, male)	+1.50	0.13
	-0.43 (n=55, female)	-0.60 (n=55, female)	+1.19	0.23
LDL-C	+0.54	+0.72	-2.11	0.03
Total cholesterol	+0.43	+0.40	+0.25	0.80

In newly diagnosed type 2 diabetic patients, fasting plasma glucose and HbA_{1c} showed very similar correlations with most of the components of metabolic syndrome except for LDL-C, which correlatedsignificantly better with HbA_{1c} than with fasting plasma glucose.

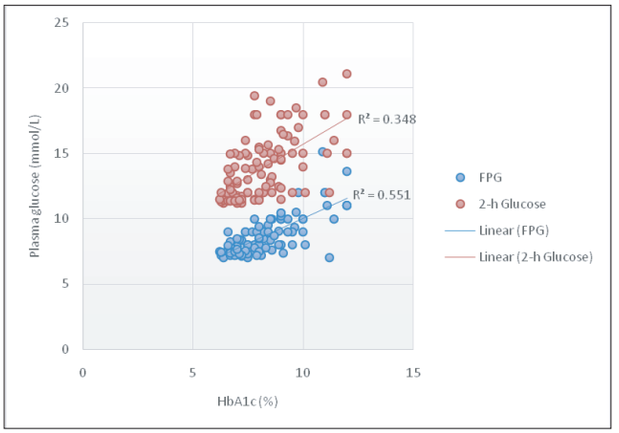

Figure 2: HbA_{1c}correlated nicely with both fasting plasma glucose (r = +0.74) and 2-h plasma glucose (r = +0.59). But the correlation between HbA_{1c} and FPG was stronger of the two.

Discussion

In this diagnostic performance study, Group A subjects were regarded as true positives and Group B subjects as true negatives for type 2 diabetes where the diagnosis was based on glucose-based WHO diagnostic criteria. We then evaluated the performance of HbA_{1c} at different threshold levels for predicting diabetes compared to the diagnosis by OGTT(Table III). Receiver Operating Characteristic (ROC) curve was constructed to determine the best cut-off level (Figure 1).

ROC curve revealed that at the WHO recommended cut-off of >6.5%, the sensitivity and specificity of HbA_{1c} for diagnosing diabetes were 95% and 93.75% respectively (AUC 0.9904) along with excellentpositive and negative predictive values. The accuracy was also very high (94.44%). Butthe highest diagnostic sensitivity (100%) and specificity (93.75%) of HbA_{1c} were seen at the cut-off of >6.15% (Table II & III, Figure 1).

In a previous study in Bulgaria, Tankova et al reported similar performance of HbA_{1c} for diagnosing diabetes (AUC0.958). HbA_{1c} cut-point of 6.5% demonstrated sensitivity of 76% and specificity of 94%. The optimal diagnostic cut-off level of HbA_{1c} was 6.1% (Sensitivity 86%, specificity 92%)[14]. In the study by Hird et al, an HbA_{1c} ≥6.5% detected diabetes with 70.3% sensitivity and 98.7% specificity (AUC 0.94) in South Africans. The optimal HbA_{1c} cut-off for detection of diabetes in that study was 6.0% (Sensitivity 89.2%, specificity 92.0%)[15]. However, in another study in Hong Kong, Yu et al noticed that an HbA_{1c} • 6.5% is highly specific (93.5%) but less sensitive (33.2%)in diagnosis of the diabetic cases.The ideal diagnostic threshold of HbA_{1c} was 6.3%with sensitivity and specificity of 56.3% and 85.5% respectively[8]. According to Kramer et al HbA_{1c} of ≥ 6.5% had 44% sensitivity and 79% specificity in diagnosing diabetes[16]. In a study in Asian Indians by Kumar et al, HbA_{1c} cut-off levelof 6.1% had optimal sensitivity and specificity[9]. Lee et al also determined HbA_{1c} threshold of 6.1% as the optimal limit for detecting diabetes in Korean adults[10]. Thus, there is considerable variation of optimal threshold of HbA_{1c} and of performance of WHO recommended cut-off. This may be due racial and ethnic differences which is known to be an important factor in influencing HbA_{1c} level[5-7]. All these differences suggest a need for different HbA_{1c} cut-off points for different populations. Generally, higher HbA_{1c} cut-off improves specificity and lower improves sensitivity. Nevertheless, our results provide justification to use HbA_{1c} to detect type 2 diabetes although the optimal cut-off is lower than WHO-recommended one.

Besides the good diagnostic performance, HbA_{1c} maintained strong correlations very similar to that of fasting plasma glucose with most of the components of metabolic syndrome in our newly diagnosed type 2 diabetic patients (Table IV). HbA_{1c} also correlated well with both fasting plasma glucose (r = +0.74) and 2-h plasma glucose (r = +0.59) in these patients (Figure 2). Tankova and colleagues observed similar correlation between HbA_{1c} level and different metabolic risk factors. In addition, significant positive correlation was established between HbA_{1c} and both fasting (r = 0.78, p<0.001) and 2-h plasma glucose (r = 0.76, p<0.001) in their study[14]. In a different study of 1,011 type 2 diabetic patients, HbA_{1c} directly correlated with cholesterol, triglycerides and LDL-C and inversely with HDL-C[17]. Correlations of HbA_{1c} with BMI, plasma glucose, weight circumference, lipid parameters and blood pressure were also established[14, 18]. In line with our results, Naveen et al showed a positive correlation between HbA_{1c} and HOMA-IR (r = 0.338, p < 0.0001), whereas Al-Hakeim found HbA_{1c} to correlate with beta-cell function in fair and poorly controlled DM[19-20]. Contrary to these findings, Borai et al observed correlation between HbA_{1c} and insulin sensitivity indices only in subjects with normal and impaired glucose tolerance but not in those with diabetes mellitus[21]. In fact, chronic hyperglycaemia is captured by HbA_{1c}, not by plasma glucose even when tests are repeated. For that reason, HbA_{1c} is a better correlate of many long-term diabetic complications like retinopathy, nephropathy and cardiovascular disease than fasting plasma glucose. Elevated HbA_{1c} is also regarded as an independent risk factor for coronary heart disease and stroke in subjects with or without diabetes[22-23].

Conclusion

In our study of healthy and newly diagnosed type 2 diabetic patients, HbA_{1c} demonstrated excellent sensitivity and specificity for diagnosing diabetes where OGTT was used as the gold standard. Due to its strong association with vascular complications, pre-analytical stability, low day-to-day variability, and convenience of sampling, HbA_{1c} can be an attractive alternative to glucose-based approach in the diagnosis of type 2 diabetes, provided that the test remains affordable and accurate.

Disclosure

All the authors declared no competing interests.

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Original Article

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Space Closure Rate in Maxillary Canine Retraction by Ni-Ti Closed Coil Spring: A Clinical Trial

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Abstract

Background: *Orthodontic treatment involving extraction of teeth is often a need to close residual space, after the initial decrowding and aligning. Pre adjusted fixed orthodontic appliances commonly utilize sliding mechanics for space closure with different types of force delivery systems. A variety of materials have been used as force delivery systems to close spaces between teeth as in the case of canine retraction after the extraction of premolars. Increasingly, nickel titanium closed coil springs are used for space closure as they are thought to retain more force over a given time period and also provide a constant force. The aim of the study was to evaluate the rate of maxillary canine retraction by Ni ti closed coil spring.* ***Materials and methods :*** *A total number of 20 patients (40 quadrants),who required canine retraction into first premolar extraction sites as part of their orthodontic treatment in the Department of Orthodontics, BSMMU as study population. The quadrants were affixed by nickel titanium closed coil springs (Medium force, Ni-Ti extension spring, Ormco) with 200gm force measured by Correx Tension Gauge. Space closure was measured by means of direct measurement from the mesial surface of mesial wing of the 2nd premolar bracket and the distal surface of distal wing of the canine bracket with digital Vernier Calipers.* ***Results:*** *Mean space closer rate in maxillary canine retraction was 1.07 ± 0.12 mm/month by Ni-Ti closed coil spring.* ***Conclusion :*** *This study revealed that space closer rate in maxillary canine retraction is 1.07 ± 0.12 mm/month by Ni-Ti closed coil spring.*

Key words

Ni-Ti Spring: Nickel Titanium closed coil spring; Canine retraction.

Introduction

Canine retraction is a very important step in treatment of patients with crowding needed first premolar extraction. In severe crowding and extraction cases, the canines have been distalized to relive the crowding as space to correctly align the incisors will not be available[1]. Among all sliding or frictionless methods of canine retraction, super-elastic nickel titanium coil spring has a particular property in producing light continuous force at a long range of action, compared with previously available materials[2-4]. The possibility that a nickel-titanium closed coil spring, with a continuous action, might have some advantages in fixed appliance space closure mechanics was investigated and compared with a currently used elastic retraction module providing an intermittent force[5-7]. Space closure via sliding mechanics can be done with various methods, but the appropriate force is applied by elastic chain or coil springs. However, the potential disadvantage of elastic chain is the significant force decay over time[4]. Nickel titanium closed coil springs have been shown to produce a constant force over varying lengths and duration, with no force decay. It may be able to meet all of the above criteria for an ideal force delivery system[8-10]. Increasingly, nickel titanium closed coil springs are used for space closure as they are thought to retain more force over a given time period and also provide a constant force[11-15]. As a result Ni-Ti closed coil springs are an alternative in wide spread use. Ni-Ti closed coil spring have reported advantage of giving significantly quicker and more constant rate of space closure[16-18]. The aim of the study was to evaluate the rate of maxillary canine retraction by Ni ti closed coil spring.

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Materials and methods

A total number of 20 patients (40 quadrants),who required canine retraction into first premolar extraction sites as part of their orthodontic treatment in the Department of Orthodontics, BSMMU as study population. Patients were selected by following these criteria, Age between 15 to 30 years who needs to 1st premolar extraction for treatment.

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Who gave consent for inclusion in the study and Free from any systemic disease. All the patients were treated with pre adjusted edge-wise fixed appliance using stainless steel 0.018x0.025 inch slot Roth brackets. After all first premolars were extracted, initial leveling and alignment was carried out. All teeth were ligated with 0.010" stainless steel litgature wire. Standardized anchorage control using tip back and toe in bend was used in all patients and 2nd premolars were included in anchorage unite. Then canine retraction carried out by individual sliding of canine using round 0.016 inch stainless steel arch wire with nickel titanium closed coil springs.The quadrants were affixed by nickel titanium closed coil springs (Medium force, Ni-Ti extension spring, Ormco) with 200gm force measured by Correx Tension Gauge. Space closure was measured by means of direct measurement from the mesial surface of mesial wing of the 2nd premolar bracket and the distal surface of distal wing of the canine bracket with digital Vernier Calipers.

Results

During this study period distribution of patients according to gender , Female were predominant. Male female ratio was 1:4. Amongst the patients, Maximum 11 (55.0%) were in age group 16-20 years followed by 7 (35.0%) and 2 (10.0%) were in age group 21-25 years and >25 years age group respectively. Mean of age was 20.60 ± 3.54 years within the range of 16-27 years.

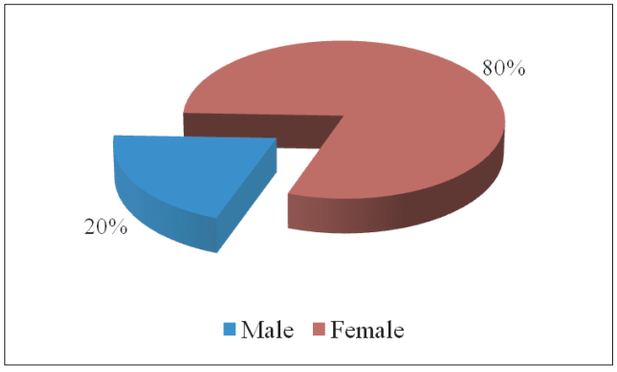

Figure 1: Pie chart of patients according to gender.

Figure 1 shows distribution of patients according to gender. Female were predominant. Male female ratio was 1:4.

Table I: Distribution of patients according to age.

Age	Frequency	Percentage
16 – 20	11	55.0
21 – 25	7	35.0
>25	2	10.0
Total	20	100.0
Mean ± SD	20.60 ± 3.54	
Range (Min – Max)	16 – 27	

Table I shows distribution of patients according to age. Maximum 11 (55.0%) were in age group 16-20 years followed by 7 (35.0%) and 2 (10.0%) were in age group 21-25 years and >25 years age group respectively. Mean of age was 20.60 ± 3.54 years within the range of 16-27 years.

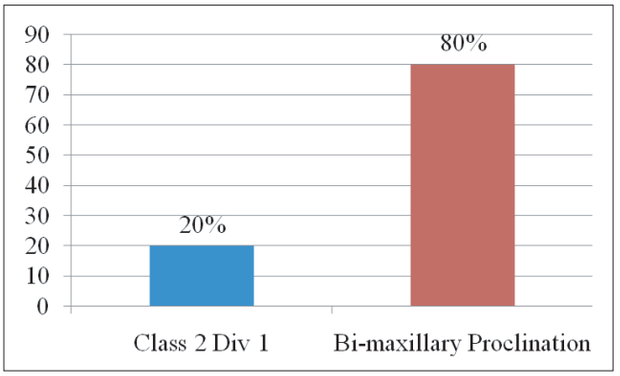

Figure 2 : Bar diagram Distribution of patients according to type of occlusion.

Figure 2 shows distribution of patients according to type of occlusion. Maximum 16 (80.0%) cases were Bi-maxillary proclination and 4 (20.0%) cases were Class 2 div 1.

Discussion

Over the period of one year from May 2015 to April 2016, this clinical trial was carried out in the Department of Orthodontics at Bangabandhu Sheikh Mujib Medical University. A total of twenty patients were selected for canine retraction.

Among all patients female were predominant with male and female ratio 1:4. According to age distribution, mean age was measured 20.60 ± 3.54 years within the range of 16-27 years. Maximum patients were in age group 16-20 years which accounted 55.0% that followed by 35.0% and 10.0% were in age group 21-25 years and >25 years age group respectively. Young populations especially female are more inclined to get a better aesthetic configuration of own self. This may be one of the causes of such type of age and sex distribution in our study. Among 17 patients in a study showed that there were 12 girls and 5 boys with a mean age of 14.7 years (Range = 11.1 to 17.1 years)[18].

In this study, a majority of 80.0% cases were Bi-maxillary proclination and the rest of 20.0% cases were Class 2 div 1.

Mean space closer rate was 1.30 ± 0.03 mm/month in male and 1.18 ± 0.12 mm/month in female in Ni-Ti closed coil spring method in maxilla.There was no statistical significant difference between these two groups.

Mean total space closer was 7.39 ± 1.07 mm in Ni-Ti closed coil spring method. In consideration of total time required for closing the space in canine retraction (Months) mean time to completing the canine retraction was 6.19 ± 0.81months in Ni-Ti closed coil spring method in maxilla.

Mean space closure rate in canine retraction was 1.19 ± 0.11 mm/month in Ni-Ti closed coil spring method in maxilla.

Conclusion

This study revealed that space closer rate in maxillary canine retraction is 1.07 ± 0.12 mm/month by Ni-Ti closed coil spring.

Disclosure

All the authors declared no competing interest.

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Original Article

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Changes of Mandibular Trabeculationin Postmenopausal Women

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Abstract

Background: *Age-related bone loss and resultant osteoporosis in a substantial proportion of the elderly population is involving a progressive loss of both bone quantity and quality. The aim of this study was to evaluate the changes of Mandibular Alveolar Bone Trabecular Pattern (MATP) according to duration of menopause.* ***Materials and methods:*** *1506 postmenopausal women were included in this study with the complain of osteoporotic like symptoms. Patient with co-morbid ailments affecting bone mineralization were excluded. Edentulous patient was also excluded from this study. All patients were evaluated by Dual X-ray Absorptiometry (DXA) for Bone Mineral Density (BMD) and Mandibular Alveolar Bone Mass (MABM) calculation.* ***Results:*** *The results of this study showed that 606 patient's had sparse mandibular alveolar bone trabeculation, 270 patient's had alternating Sparse and dense, 630 patient's had Dense MATP. Chi-square test, the p-value was less than 0.05 in Osteoporotic fracture with menopausal time and osteoporotic fracture in different MATP.* ***Conclusion:*** *There was a significant relation between Osteoporotic fracture and Menopausal time, MATP and Osteoporotic fracture.*

Key word

Bone Trabeculation; Post-menopause; Osteoporosis; Microstructure.

Introduction

The proportion of elderly person in the world population has increased substantially and will continue to do so in the coming years. Aging has multiple complex effects that result in the progressive deterioration of various organ, including the skeleton. Age related bone loss and osteoporosis in the elderly increase the risk of fractures and morbidity in this population[1]. Osteoporosis is a common disease of post-menopausal women, characterized by low bone mass and microstructural deterioration of bone tissue, with an increases fracture risk[2,3]. With 2.68 million new broken bones every year in the EU6, fragility fracture are a major obstacle to healthy aging, impacting the independence and quality of life of 20 million women and men living with osteoporosis[4].

A large proportion of the population visit their dentist annually, and dental radiographs are routinely taken. Image perception is an important aspect of diagnostic imaging[5]. In dental radiography, radiographs show bone a radiographic trabecular pattern, an irregular meshwork of vague bright lines with fuzzy dark meshes[6]. Trabecular bone structure can be assessed by measuring trabecular volume, spacing and connectivity by computed tomography and magnetic resonance[7]. The intra-oral and panoramic radiographs provide information about the mandibular and maxillary bone at low cost to the patient and without undue exposure to radiation[8]. The alveolar process of the mandible develops as a result of the tooth eruption and elongation. It is subject to physiologic remodeling throughout life, and can be influenced by masticatory demands, orthodontic movements and may undergo more or less progressive resorption after tooth extraction. The mandibular alveolar process undergoes the same aging processes as other bones and thickness of the trabeculae, spacing between that trabeculae, and the trabecular connectivity in the jaw are altered in patients with osteoporosis compared to normal subjects[9,10].

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From the time of attainment of peak bone mass, there is a decrease in trabecular bone volume with aging in both sexes, although not at all sites and not uniformly for males and females[11]. Trabecular bone architecture changes according to the loading history of the individual, there are others factor such as nutrition, co-morbidities, social activities and work activities that affect bone metabolism, independent of direct mechanical stimuli[12]. In the females, changes in the trabecular bone are most evident at and after the menopause, which is associated with decrease estrogen[13]. The greatly increased activation of osteoclasts associated with decrease estrogen in menopausal females results in an imbalance between resorption and formation with a consequent net bone loss[14].

The alveolar trabeculation was classified as either sparse, mixed dense plus sparse or dense[11]. When the trabecular pattern was sparse, the criterion was large intertrabecular spaces in most of the alveolar process, especially in the cervical, dentate, premolar area. When the trabecular pattern was evaluated as dense, the whole radiographed alveolar premolar area had small intertrabecular spaces. When the trabecular pattern was assessed as mixed dense plus sparse, the trabeculation was mostly dense cervically and sparse apically[15].

Age-related bone loss and resultant osteoporosis in a substantial proportion of the elderly population is multifaceted and multifactorial, involving a progressive loss of both bone quantity and quality. Age related microstructural changes in bone are complex. The most important step is trabecular bone loss among the three major age-related processes that lead to bone loss. The decrease in trabecular bone is caused by thinning of the trabeculae and especially in early postmenopausal women, by disruption of the trabecular microstructure and loss of trabecular elements[16]. The aim of this study was to evaluate the changes of mandibular trabecular pattern according to duration of postmenopausal condition.

Materials and methods

1506 Postmenopausal women were included in this study with the complain of osteoporotic symptoms. Patient with co-morbid ailments affecting bone mineralization were excluded. Edentulous patient was also excluded from this study. All patients were evaluated by DXA for BMD and Digital Dental Radiograph for mandibular alveolar bone trabecular pattern (MATP) and Mandibular Alveolar Bone Mass (MABM) calculation. All patient was divided into three group according to duration of menopause named i) Menopausal time <20 years ii) Menopausal time 20-40 years iii) Menopausal time >40 years. On the other hand, Mandibular trabecular pattern was divided in three named i) Sparse ii) Sparse and Dense alternate iii) Dense.

BMD (Bone Mineral Density): DXA (Dual-energy X-ray absorptiometry) is a low radiation X-ray capable of detecting quite small percentages of bone loss. It is used to measure spine and hip bone density and can also measure bone density of the whole skeleton. The world health Organization has defined a number of threshold values (Measurement) for osteoporosis. The reference measurement is derived from bone density measurements in a population of healthy young adults (called a T-score). Osteoporosis is diagnosed when a person's BMD is equal to or more than 2.5 standard deviation below this reference measurement.

Mandibular Trabecular Pattern: A digital radiograph (Gendex sensor, Vixwin software, USA) of the premolar region was obtained using a standardized paralleling technique. All radiographs were done in same procedure. The overall trabecular pattern (Trabeculation) was assessed using a visual index modified by Jonasson. Only dentate patient was included and the trabecular pattern was assessed avoiding areas with recently extracted teeth and bone around the fixed partial denture. With the help of these radiographs, the alveolar trabeculation was classified as either as sparse, alternating dense and sparse and Dense. When the trabecular pattern was evaluated as sparse the criterion was large inter-trabecular spaces especially in premolar area (Figure 4). When the trabecular pattern was evaluated as dense the entire radiographed area had an equal degree of trabeculation and the inter-trabecular spaces were small even under the roots (Figure 5). When the trabecular pattern was assessed as alternating dense and sparse trabeculations were normally denser cervically and sparse apically (Figure 6). When it was difficult to classify the trabecular pattern, alternating dense and sparse was chosen.

Mandibular Alveolar Bone Mass (MABM): The MABM by the mean gray level values of the alveolar bone on the digital radiograph. The Region of Interests (ROIs) were set on the apical radiograph of the individual on the 6 mm step of the reference radiograph with 'rectangular tool' avoiding the lamina dura and the most crestal location. No apical bone was included in ROIs. Then the Pixel Intensity (PI) was measured from low level to high level (Assigning the

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value 0 to black 256 to white). Areas of bone loss represent as darker while areas of the bone gain or dense bone as lighter areas. MABM was evaluated from the mean value of PI. This obtained data are presented as mean ± Standard Deviation (SD) and it was posted to the data sheet for statistical analysis.

All collected data were posted in preformed data sheet according to Good Clinical Practice (GCP). Calculation of mean and standard deviation was performed by ANOVA using SPSS 11.5. Chi-square test was performed for evaluate the relation of change of mandibular trabecular pattern with menopausal time, Osteoporotic fracture with menopausal time and Osteoporotic fracture in different mandibular alveolar trabecular pattern. A p-value <0.05 was considered as statistically significant.

Results

Total 1506 postmenopausal women were included in this study. Among those, 606 patient's had Sparse Mandibular Trabecular Pattern (MATP) 270 patient's had alternating Sparse and Dense, 630 patient's had Dense Mandibular Trabecular Pattern (MATP).

Baseline Characteristics of Patient: The mean age of Patient was 61.8 ±11.3, 58.3 ±9.69, 60.8 ±11.2 year in Sparse, Alternating Sparse and Dense, Dense MATP respectively (Table-I). The mean height of patient was 147.28 ±8.19 cm in Sparse MATP, 149.06 ±6.33 CM in alternating Sparse and Dense MATP, 149.24 ±8.54 cm in Dense MATP (Table-I). The mean Weight of Patient in Sparse MATP was 49.88 ±10.65, 52.61 ±10.05 kg in alternating Sparse and Dense MATP, 51.69 ±11.17 kg in Dense MATP (Table-I). T score of Lumber BMD in Sparse MATD patient was -3.15 ±1.30gm/cm^{2}in alternating sparse and dense MATP t-score of lumber BMD was -2.57 ±1.25 gm /cm^2 2.70 ±1.32 gm/cm^{2}in Dense MATP. T score for Femoral neck BMD in Sparse MATP was -3.47 ±1.57 gm/cm^2 -2.72 ±1.32 gm/cm^{2}in alternating Sparse and Dense MATP, -2.84 ±1.38 gm/cm^{2}in Dense MATP (Table- I). In sparse MATP, minimum to maximum Alveolar bone mass was 62.85 ±24.84 to 94.52 ±26.49; In alternating sparse and dense MATP, minimum to maximum alveolar bone mass was 67.82 ±21.08 to 102 ±43.43; in dense MATP, minimum to maximum alveolar bone mass was 80.44 ±24.22 to 111.62 ±23.22 (Table-I).

Change of MATP with Menopausal Time: Total 1148 (76%) women had the history of menopausal time <20 years, 340 (22.6%) patient had the history of menopausal time about 20 to 40years, 16 (1.1%) of patient's had the history of menopausal time >40 years (Fig 1). Sparse MATP was find 36.8% (n=423) of patient, Alternating Sparse and Dense MATP was find 19.4% (n=223) of patient, Dense MATP was find 43.70% (n=502) 0f patient those who's menopausal time <20 years (Fig 1) . 50.6% (n=172) of patient had Sparse, 13.8% (n=47) of patient had alternating Sparse and Dense, 35.6% (n=121) of patient had Dense MATP with the history of menopausal time 20-40 years (Fig 1). 56.3% (n=9) of patient had Sparse and 43.8% (n=7) of patient had Dense MATP those who's menopausal time >40 years (Fig.1). In Chi-Square test, Pearson Chi-square value was 25.027, likelihood Ratio was 27.572 and Degree of Freedom (df) was 4. The p-value was less than our choices significance level p=0.05, so we can conclude that there was a significant association between Mandibular Alveolar trabecular Pattern and Menopausal time (Table-II).

Osteoporotic Fracture with Menopausal Time: Total 23.5% (n=353) of patient had osteoporotic fracture, 76.41% (n=795) of patient was Non-fractured, those who involved in this study. Among those 18.2% (n=208) of osteoporotic fractured patient's menopausal time was <20 years, 40.3% (n=137) of patient's menopausal time 20-40 years, 50% (8) of patient's menopausal time > 40 years (Fig 2). From non-fractured involving patient, 81.8% (n=584) had menopausal time <20 years, 59.7% (n=203) had menopausal time 20-40 years, 50% (n=8) had menopausal time > 40 years (Fig 2). In Chi-square test, Pearson Chi-square value was 78.019, Likelihood Ratio value was 72.141, and linear-by-liner Assoc. value was 76.798. Degree of freedom for Pearson Chi-square and Likelihood ration was 4, Linear-by-linear Assoc. was 1 (Table- III). The p value was less than our choices significance level p=0.05, so we can conclude that there was a significant association between Osteoporotic fracture and Menopausal time (Table III).

Osteoporotic Fracture in Different MATP: 30.6% (n=185) of Sparse MATP patient's had osteoporotic fracture and 69.3% (n=795) was non-fractured. In alternating sparse and dense MATP, 17% (n=46) had osteoporotic fracture and 83%(n=224) was non-fractured. 19.6% (n=123) of Dense MATP had osteoporotic fracture and 80.4%(n=504) was non-fractured (Fig 3). In Chi-square test, Pearson chi-square value was 30.226, Likelihood ratio value was 30.301 and Degree of freedom (df) was 4. The p value was less than our choices significance level p=0.05, so we can conclude that there was a significance association between MATP and Osteoporotic fracture (Table IV).

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Table I: Characteristics of Osteoporosis women in different Mandibular Trabecular Patter (MATP).

Description	Sparse (n=606)	Sparse & Dense (n=270)	Dense (n=630)
Age (year)	61.8 ±11.3	58.3 ± 9.69	60.8 ± 11.2
Hight (CM)	147.28 ±8.19	149.06 ±6.33	149.24 ± 8.54
Weight (kg)	49.88 ± 10.65	52.61 ± 10.05	51.69 ± 11.17
T score for Lumber spine BMD (gm/cm^2)	-3.15 ± 1.30	-2.57 ±1.25	-2.70 ±1.32
T score for femoral neck BMD(gm/cm^2)	-3.47 ± 1.57	-2.72 ± 1.32	-2.84 ± 1.38
Alveolar Bone mess (minimum)	62.85 ± 24.84	67.82 ± 21.08	80.44 ± 24.22
Alveolar Bone mess (Maximum)	94.52 ± 26.49	102.99 ± 43.43	111.62 ± 23.22

Table II : Changes of Mandibular Trabecular Pattern with Menopausal time.

Description	Menopausal time <20 years. n=1148 (76%)	Menopausal time 20-40 years. n=340(22.6%)	Menopausal time >40 years. n=16(1.1%)
Sparse MATP. n=604(40.2%)	423 (36.8%)	172 (50.6%)	9 (56.3%)
Sparse and Dense MATP. n=270(18%)	223 (19.4%)	47 (13.8%)	0(0%)
Dense. n=630(41.9%)	502 (43.70%)	121 (35.6%)	7(43.8%)
Chi-Square Test			
	Value	df	Asymp. Sig (2-sided)
Pearson Chi-square	25.027[a]	4	.000
Likelihood Ratio	27.572	4	.000
n of Valid Cases	1504		

a. 1 cells (11.1%) have expected count less than 5. The minimum expected count is 2.87.

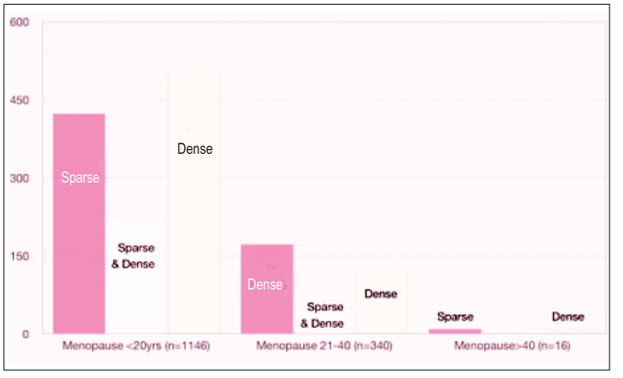

Figure 1 : The Changes the MATP within the menopausal time of osteoporotic women.

Table III: Osteoporotic fracture with menopausal time.

Description	Menopausal time <20 years. n=1148 (76%)	Menopausal time 20-40 years. n=340(22.6%)	Menopausal time >40 years. n=16(1.1%)
Osteoporotic Fracture n=353(23.5%)	208 (18.2%)	137 (40.3%)	8(50%)
Non-fracture n=795(76.41%)	584(81.8%)	203 (59.7%)	8 (50%)
Chi-Square test			
	Value	df	Asymp. Sig (2-sided)
Pearson Chi-square	78.019[a]	4	0.000
Likelihood Ratio	72.141	4	0.000
Linear-by-liner Assoc.	76.798	1	0.000
n of Valid Cases	1502		

a.4 cells (44.4%) have expected count less than 5. The minimum expected count is .01.

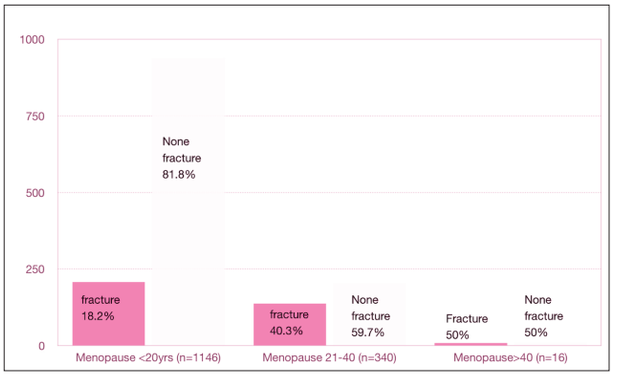

Figure 2 : Osteoporotic fracture and menopausal time

Table IV : Osteoporotic fracture in different MATP of osteoporotic women.

Description	Osteoporotic fracture n=354 (23.5%)	Non-fracture. n=795 (76.4%)
Sparse. n=605(40.2%)	185 (30.6%)	419(69.3%)
Sparse & Dense. n=270(18%)	46 (17%)	224(83%)
Dense. n=629(41.8%)	123(19.6%)	504(80.4%)
Chi-Square test		
	Value	df	Asymp. Sig (2-sided)
Pearson Chi-square	30.226[a]	4	.000
Likelihood Ratio	30.301	4	.000
N of Valid Cases	1504		

a. 3 cells (33.3%) have expected count less than 5. The minimum expected count is 0.18

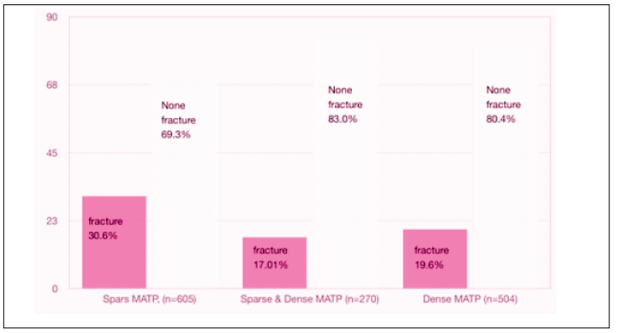

Figure 3 : Osteoporotic fracture with Mandibular Alveolar Trabecular Pattern (MATP).

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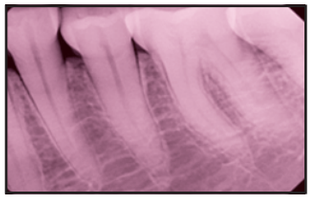

Figure 4 : Sparse MATP

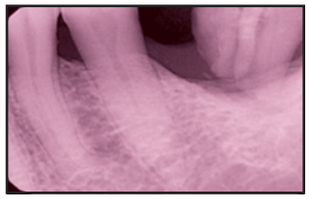

Figure 5 : Alternating Sparse and Dense

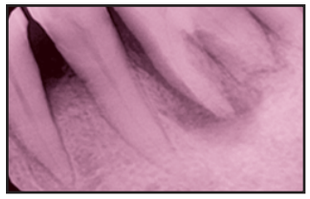

Figure 6 : Dense MATP

Discussion

The age-related bone loss and resultant osteoporosis in a substantial proportion of elderly population is multifaceted and multifactorial, involving a progressive loss of both bone quality and quantity. Osteoporosis is a common disease of elderly, characterized by low bone mass and micro-structural deterioration of bone tissue, with increased fracture risk[16]. The case of osteoporosis, oral bone loss may be age-related because of poorer nutrition, alternations in dental hygiene, and hormonal changes[17]. The mean of age of Sparse MATP patient was 61.8 ±11.3 years more than alternating Sparse and Dense (58.3 ±9.69) and Dense (60.8 ±11.2) mandibular Trabecular Pattern. In Sparse MATP, the height (147.28 ±8.19) and Weight (49.88 ±10.65) was less than alternating sparse and dense MTP height (49.06 ±6.33) and Weight (52.61 ±10.05), also lower than Dense MATP's Height (149.24 ±8.54) and weight (51.69 ±11.17).

All patients those were include in this study diagnosed as Osteoporosis by DXA scan of lumber spine and femoral neck. Osteoporosis diagnosis and staging are based on the identification of different risk factors, the most important being low Bone Mineral Density (BMD) of femoral neck and lumber spine[18]. World Health Organization (WHO) has been established four diagnostic level of BMD: i) The normal bone when t score is better than -1. ii) Osteopenia when t score is between >1 and 2.5 iii) Osteoporosis when t score is < -2.5 (4). Established osteoporosis when non traumatic fractures are includes and t score is <-2.5[19]. In our study, sparse MATP patient's, T score for lumber BMD was -3.15 ±1.30 and femoral BMD was -3.47 ±1.57. Sparse MATP patient's lumber and femoral BMD was lower than alternating sparse and dense MATP patient (-2.57 ±1.25 & -2.72 ±1.32) and Dense MATP patient (-2.70 ±1.32 & -2.84 ±1.38).

The Mandibular Alveolar Bone Mass (MABM) in this study was minimum 62.85 ±24.84 to maximum 94.52 ±26.49 for sparse MATP patients. The MABM of sparse MATP patient had lower than alternating sparse and dense MATP (67.82 ±21.08 to 102.99 ±43.43) and dense MATP (80.44 ±24.22 to 111.62 ±23.22). In other study showed the mandibular alveolar mass of normal postmenopausal patient were significantly different from osteopenic and osteoporotic patient[20]. The method for estimating MABM by its optical density is obsolete. The deficit in bone formation in cortical bone results in an increased number of lacunae and porosities and later on in thinner cortical plates, it leads to larger intertrabecular spaces and a thinning of the trabeculae in cancellous bone[21,22]. When the cortices and trabeculae are thinner and the intertrabecular spaces are larger in a certain area (ROI) the mineral content is decreased, and therefore, also the grey-level value of the radiographed area is decreased.

Age-related microstructural changes in bone are complex. There are three major age-related processes that lead to bone loss. The first and most important is trabecular bone loss. The first and most important is trabecular bone loss. The decrease in trabecular bone is caused by thinning of the trabeculae and, especially in early postmenopausal women, by disruption of the trabecular microstructure and loss of trabecular elements[16]. 56.3% (n=9) of patient with menopausal time >40 years had shown the Sparse MATP, 36.8% of patient with menopausal time <20 years, 50.6% of patient with menopausal time 20-40 years. In chi-square statistical analysis showed that the p value was less than 0.05. This finding proved that increased the number of lacunae and porosities, it leads to larger intertrabecular spaces and thinning of the trabeculae with increasing age and increase the menopausal time of osteoporotic women. There was a significance association between MATP and postmenopausal time.

During life, mammalian bone undergoes a process of continuous remodeling in which old bone is resorbed and replaced with newly formed bone. In overall amounts of resorbed and formed bone are balanced. With aging, however, this remodeling balance becomes negative, resulting in a decreased bone mass. The decline bone mass is associated with reduced bone strength, resulting osteoporosis and risk of non-traumatic fracture risk[23]. The effects of age on the fracture risk could be due to a number of factors, including bone microstructure deterioration and possible changes in bone material properties, such as the

composition and degree of collagen cross-linking[16]. 50% (n=8) of patient with osteoporotic fracture had menopausal time more than 40 years, contrariwise only 18.2% (n=208) had less than 20 years menopausal time and 40.3% (n=137) of patient had 20 to 40 years menopausal time. In chi-square test, the p value was less than 0.05. So, there was a significant association between osteoporotic fracture and menopausal time.

Although osteoporosis is associated with a high fracture risk, roughly 73% of fractures occur in women who test negatively for osteoporosis[24]. Bone structure is now considered the key to understanding fracture risk[25]. Trabecular bone structure can be assessed by measuring trabecular volume, spacing, and connectivity by computer tomography and magnetic resonance[26]. Mentionable, trabecular bone structure can be visually evaluated even in plain dental radiographs. In this study, 30.6% (n=185) of Osteoporotic fracture involved menopausal women had Sparse MATP. On the other hand, 80.4% (n=504) of non-fracture involved menopausal women had Dense MATP. The p-value in chi-square test was less than 0.05, that means significant association between osteoporotic fracture and MATP. Another study also found that trabecular pattern was a highly significant predictor of future risk both in perimenopausal and in older women: the older individual, the more effective the mandibular trabecular pattern as a fracture predictor[27,28]. 20% of 38-54 years old women in same study had sparse trabeculation. But in our study, almost same age group had 30.6% postmenopausal women had fracture in sparse MATP.

Conclusion

Changes in the mandibular alveolar bone trabeculation reflect alternations in the skeletal BMD, they can be observed on digital dental radiograph. Menopausal time and Mandibular Alveolar Bone Trabecular Pattern (MATP) are significantly related with identify women at high risk for non-traumatic fracture in postmenopausal condition, often many years before the first fracture occurs.

Disclosure

All authors declared no competing interest.

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Original Article

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Accuracy of FNAC in the Detection of Metastatic Lymphnodes in Oral Squamous Cell Carcinoma

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Abstract

Background : *Bangladesh accounts for the majority of oral cancer cases occurring worldwide. The metastasis of oral cancer to the regional lymph nodes and distant sites determines the prognosis and the survival rate of this disease.The objectives of this study were to evaluate the accuracy of preoperative clinical FNAC in comparison with postoperative histopathological findings in determination of metastatic cervical lymph nodes and also to assess whether combining these techniques increases the specificity and sensitivity of lymph node metastasis in oral Squamous Cell Carcinoma (SCC).* ***Materials and methods :*** *It is a descriptive type of cross sectional study conducted at the Department of Oral and Maxillofacial surgery in Bangabandhu Sheikh Mujib Medical University (BSMMU) Dhakafrom 1st September 2011 to 31st August 2011. Total, 30 consecutive biopsy proven cases of oral SCC were included and the nodal status was evaluated by preoperative FNAC and confirmed by histopathological examination.* ***Results :*** *The results were presented in terms of sensitivity, specificity, predictive valuesand predictive accuracy. It was found that sensitivity, specificity, positive predictive value, negative predictive value and predictive accuracy of the preoperative FNAC reports in comparison with post operative histopathology reports were 93.1%, 83.34%, 90.9%, 89.47%, 90% respectively.* ***Conclusion :*** *Preoperative FNAC of neck nodes associated with oral SCC provides a better assessment of the neck for nodal metastasis and make a significant impact on treatment modalities*

Key words

Fine Needle Aspiration Cytology (FNAC); Lymph node; Squamous Cell Carcinoma (SCC).

Introduction

Oral cancer is used to describe any malignancy arise from oral soft and hard tissues. Among all malignancies of oral cavity Squamous Cell Carcinoma (SCC) is the most common malignant growth involves the oral cavity and shows significant degree of metastasis.It contributes approximately 90% of oral malignancies[1]. SCCcauses major health problem in developing countries, representing a leading cause of death. Tobacco is themain aetiological factor of oral cancer in Bangladesh[1,2,3]. SCC is one of the most dominant cancer in the world according to the World Health Organization (WHO)[4]. Oral cancer mostly affects in the area of lips, tongue, mouth, salivary glands and oropharynx[4,5,6]. In Bangladesh more than 7000 people are newly diagnosed each year and among them 6.6% people are died due to their life style and other factors[2,3,5,6].

Squamous Cell Carcinoma always invades the surrounding structures. It eventually enters the channels like the lymphatics and blood vessels and group of cells are carried to the other parts where it sets up as anabolic spread, is called metastasis. If it is confined to the lymphnode then it is termed as nodal metastasis.The tumor can metastasise in ipsilateral or contralateral or bilateral neck nodes. As the tumor grows within a node, it enlarges and becomes indurate and rounded. The tumor eventually extends though the capsule of the lymph node and invades the surrounding structures, extension through the neurovascular bundle is relatively common and results its fixation.Therefore preoperative assessment of regional lymphnodes is most essential step for performing adequate treatment to enhance the life expectancy of the patients having oral Squamous Cell Carcinoma. The study protocol was explained to the patients in detail before obtaining the informed

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consent from the patients. Patients were allocated randomly for the study. Main objective of this study to specify FNAC as an effective diagnostic tool to determine metastatic cervical lymphnodes in oral squamous cell carcinoma.A number of good studies have been published about the accuracy of FNAC to detect metastastic lymphnodes in oral SCC carried out in a number of training institutes of certain countries all around the world, but none from department of Oral and Maxillofacial surgery, Bangabandhu Sheikh Mujib Medical University, Bangladesh.

FNAC can improve the examination of cervical lymphadenopathies in patients with oral SCC and provides reliable information about the extent of the disease and adequate planning for therapeutic approach. Therefore, FNAC can be a suitable substitute for lymphnode open biopsy and able to resist patients from surgical hazards.

Materials and methods

This descriptive type of cross sectional study was done to evaluate the accuracy of preoperative clinical FNAC in comparison with postoperative histopathological findings in determination of metastatic cervical lymph nodes and also to assess whether combining these techniques increases the specificity and sensitivity of lymph node metastasis in oral Squamous Cell Carcinoma (SCC).

The study was carried out in Oral and Maxillofacial Surgery Unit of Bangbandhu Sheikh Mujib Medical University (BSMMU) Dhaka from 1st September 2011 to 31st August 2011. Total 30 patients who were diagnosed of having oral SCC with palpable regional lymph nodes treated in the department of Oral and Maxillofacial Surgery, BSMMU during the study period and, meeting the inclusion and exclusion criteria were included randomly for the purpose of this study. Patient having histologically determined oral SCC with palpable cervical lymphnodes are included in the study. Those having advance state of oral SCC which was inoperable and prone for distant metastasis were excluded. All the data were collected in a standardized data collection sheet. Data were collected from a total of 32 patients with a clinical diagnosis of SCC with palpable lymphnodes. Of those, data of 2 patients were not included in the analysis (Clinically inoperable and eventually referred to Department of Oncology for palliative care). Therefore data of a total of 30 patients were analyzed for the purpose of the study. The study protocol was explained to the patients in detail before obtaining the informed consent from the patients. All the data were collected in a standardized data collection sheet. They were screened and checked for any missing value and discrepancy. The data were then analyzed by using Statistical Package for Social Sciences (SPSS). Data were intercepted accordingly and presented in tables, charts and bar diagrams.

Results

Figure-1 showed that the highest number of the respondents (n=25) were in the age group of years > 40 years (83.3%). Mean age of the patients were 50.83 years.Other patients were in age group < 40 years (n=5) (16.7%).Figure-2 showed that female respondent were slightly more than male respondent which was 56.7% (n=17) female and 43.3% (n=13) was male. Table-I showed that 56.67% (n=17) of the respondents were house wives, 20.00% (n=6) were businessman, 13.33% (n=4) were service holders and rest of the respondents were workers 10.00% (n-3).Table-II demonstrated the diagnostic accuracy of preoperative FNAC in compare with postoperative histopathology in the detection of metastatic lymphnodes. Out of 30 patients (n-30), 27 (90.00%) patients FNAC reports show accurate results, remaining 3 patients (10.00%) FNAC reports showed inaccurate results.Figure-3showed that out of 30 patients undergo for this study shows 33.3% (n-10) showed positive results, 56.7% (n-17) showed negative results, 6.7% (n-2) showed false negative results and 3.3% (n-1) showed false positive results by comparing both preoperative FNAC results with postoperative histopathology reports of suspicious lymphnodes. It was found that sensitivity; specificity, positive predictive value, negative predictive value and predictive accuracy of the FNAC reports in comparison with postoperative histopathology reports were 93.10%, 83.34%, 90.90%, 89.47% respectively (Table-III). Figure-4 showed the pattern of primary sites of tumour. It was found that highest percentage were in the buccal mucosa (60.0%) followed by labial mucosa (13.3%) alveolar mucosa & vestibule (10.0%) andretro molar trigone (16.7%). Figure-5 showed the percentage of primary involvement side present among the study population. Out of 30 patients 60% having primary lesion as well as nodal involvement towards the right side and 40% towards the left side.Table-IV showed that based upon TNM grading 76.7% (n-23) were categorized in Grade I, 20.0% (n-6) were categorized in Grade II & remaining 3.3% (n-1) was categorized in Grade III.Table V showed that 53.33% (n=16) of the respondents were from poor socioeconomic class, 40.00% (n=12) were from middle class and 6.67% (n=2) were from rich class.

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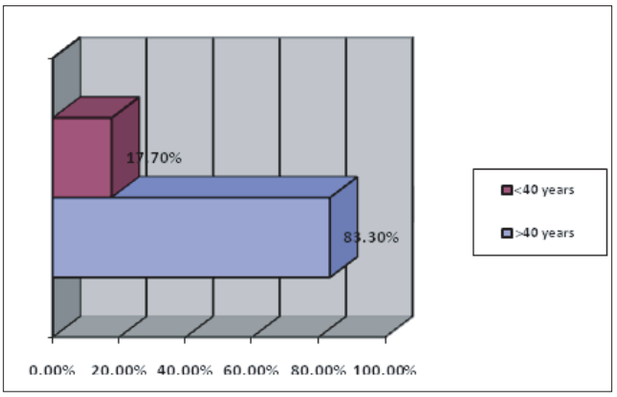

Figure 1 : Distribution of the respondents according to their age (n=30).

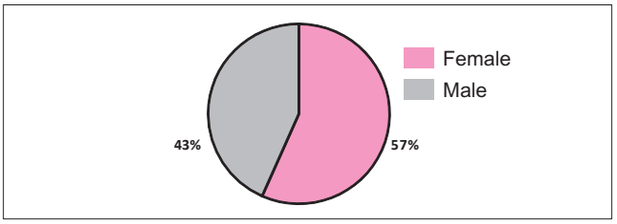

Figure 2 : Distribution of the respondents according to their sex (n=30).Out of the respondents 56.7% (n-17) patients are female, remaining 43.3% (n-13) are male.

Table I : Distribution of the respondents according to their occupation (n=30).

Types of Occupation	Number	Percentage
House wife	17	56.67
Business	6	20
Service	4	13.33
Worker (Construction/ Machinery)	3	10
Total	30	100

Table II : Diagnostic accuracy of preoperative FNAC in comparison with postoperative histopathological reports in detection of metastatic lymph nodes in Oral SCC(n=30).

Diagnostic Accuracy	Number of the patients	Percentage (%)
Accurate	27	90%
Inaccurate	3	10%
Total	30	100%

Figure 3 : Status of the results comparing preoperative FNAC with post operative histopathology (n=30).

Table III : Distribution of findings between preoperative FNAC and postoperative histopathology (n=30).

Sensitivity	93.1%
Specificity	83.34%
Positive predictive value	90.9%
Negative predictive value	89.47%

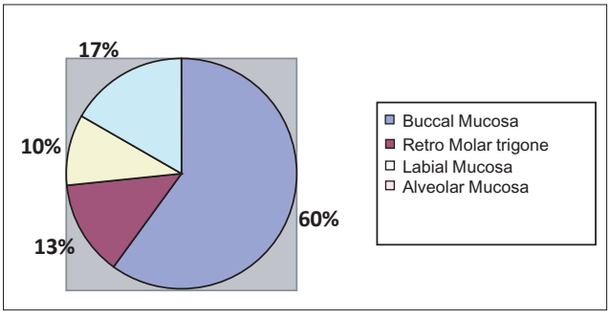

Figure 4 : Distribution of the respondents according to the primary sites of tumor involvement (n=30). Among the distributions, pattern of involvements are Buccal Mucosa 60% (n-18), Retro Molar trigone 17% (n-05), Labial mucosa 13% (n-04) and Alveolar mucosa 10% (n-03) respectively.

Table IV : Distribution of the study patients according to TNM Grading (n=30).

TNM Grading	No of patients	Percentage (%)
Grade I	23	76.7
Grade II	6	20
Grade III	1	3.3
Total	30	100.0

Table V : Distribution of the respondents according to socioeconomic status (n=30).

Socioeconomic status	Number	Percentage (%)
Poor	16	53.33
Middle class	12	40
Rich	2	6.67
Total	30	100

Discussion

Out of 30 patients who underwent for preoperative FNAC of suspicious lymphnode and successively went through margin free excision along with neck dissection, show 90.00% accuracy of FNAC in compare with lymph node histopathology postoperatively. The finding was quite similar to showed in their study that preoperative accuracy of FNAC is 90.00% out of their 50 respondents[7,8].

In this study shows that the ratio of false positive and negative result of preoperative FNAC in compare with postoperative lymph node histopathology are 3.3% & 6.7% respectively which is relatively close to that of

showed in their study in which ratio of false positiveand negative result of preoperative FNAC in compare with postoperative lymph node histopathology are 4.1% and 2% respectively out of their 52 study population[9].

Present study showed that sensitivity and specificity of preoperative FNAC were 93.10% and 83.34% respectively, which was quite identical as that of showed in their study[9-11]. Current study showed that incidence of primary site preference oral squamous cell Carcinoma was buccal mucosa (60%), labial mucosa (13.3%), alveolar mucosa and vestibule (10%) and retro-molar trigone (16.7%) respectively which relatively close with that of[4,12].

Current study showed that among study population was also subdivided based upon their TNM grading which reveals that 76.7% belongs to Grade I-20% belongs to Grade II -20% and remaining of study population belongs to Grade III -3.3%, which is quite similar to[13].

Current Study showed Most of the respondents belongs to poor community, which is 53.3% (n-16). Present study shows some extent of similarity with that of [14].

Conclusion

The present results showed a similar frequency of accuracy of FNAC in detection of metastatic lymphnodes in oral SCC when compared to other similar studies. According to the study diagnostic accuracy of FNAC was obtained 90.00%. Key aspects of this study are ensuring early and adequate detection of metastaticlymph nodes in order to provide adequate management to the patient and establishment of diagnostic value of preoperative FNAC of suspicious lymph nodes. It is recommended that a detail history, thorough clinical examination, radiological examination are mandatory for the diagnosis and better management of metastatic lymph nodes in oral Squamous Cell Carcinoma. FNAC is a rapid, safe, easy and non-expensive diagnostic technique which can be used for initial diagnosis of metastatic lymphadenopathy.

Disclosure

All the authors declared no competing interest.

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Review Article

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Human Amnion Membrane: The Magic Membrane

Niaz Ahmed[1*] Md. Ali Hossain[2] Md Kamrul Hasan[3]

Abstract

The amnion membrane is the innermost lining of the placenta. The amnion membrane has natural biologic properties that prevent inflammation, reduce scar formation, prevent abnormal blood vessel formation, and promote wound repair, healing and re-epithelialization as well as pluripotent, non-antigenic properties. Amnion membrane has been also used as an allograft in general surgery for reconstruction, as an auto graft in neonatal reconstruction surgery, and as a scaffold in tissue engineering research. The aim of this review was to evaluate the technique of clinical application of human amnion in dentistry.

Key wards

Amnion Membrane; Tissue engineering; Dentistry.

Introduction

Greek name suggests 'Amnion'-as a membranous sac that contains the conceptus and the amniotic fluid.It is an adjustable bio-container that provides the fetus a limited space to allow movements. The amnion is a metabolically active membrane that is involved in solute and water maintaining amniotic fluid homeostasis[1,2].

The amnion membrane is the innermost lining of the placenta and consists of a thin epithelial layer on a thick basement with an avascular stroma (Fig. 1). There are no nerves, muscle fibers, or lymphatics in the amnion. In the amniotic epithelium, the specialized arrangement of intercellular cytoskeletal filaments such as actin, alphaactin, spectrin, ezrin, cytokeratins, vimentin, and desmoplakin indicates their role in the structural integrity and modulation of cell shape as well as in the junctional permeability. Laminin is one of the main components of the basement membrane and it critically contributes to cell differentiation, cell shape and movement, maintenance of tissue phenotypes, and promotion of tissue survival via cell surface receptors such as integrins and dystroglycans (Fig 1).

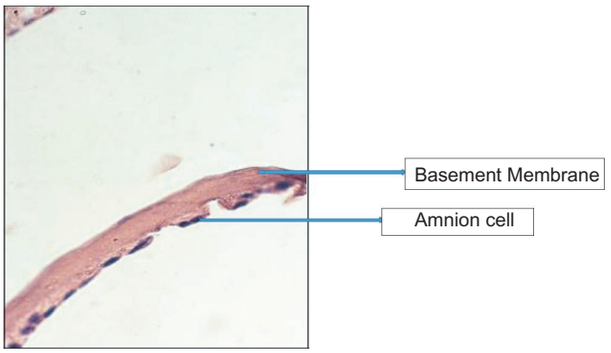

Fig. 1 Amniotic membrane

The amnion membrane has natural biologic properties that prevent inflammation, reduce scar formation, prevent abnormal blood vessel formation, and promote wound repair, healing, and re-epithelialization as well as pluripotent, non-antigenic properties. It is inexpensive and readily available in large amounts.

HAM contains two cell types, from different embryological origins, which display some characteristic properties of stem cells. Human Amnion Epithelial Cells (hAECs) are derived from the embryonic ectoderm, whereas human Amnion Mesenchyme Stromal Cells (hAMSCs) are derived from the embryonic mesoderm. Both populations have similar immunophenotype and multi-potential for in-vitro differentiation into major mesodermal lineages. The amniotic membrane secretes nutritious factors and suppresses the semi-allogeneic immune response against the fetus[3-6]. The thickness of the human term amnion varies among individuals and depends on the location of the sample

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(70–180 m thick), however, it is remarkably strong and elastic. Amnion withstands the progressive stretching of the growing embryo, internal and external traumas, and fast and slow pressure changes. In a vast majority of the amniotes, amnion is one of the very few tissues that has no vascularity. In humans, the chorion and the amniotic fluid transfer nutrients to the avascular amnion by diffusion[7]. Amniotic membrane has an excellent candidature to be used as a native scaffold for tissue engineering, and in addition, maybe easily obtained, processed and transported[8].

Amniotic membrane is a semipermeable membrane and is an immune-tolerant structure. The amniotic membrane fulfills the current mechanical concept of Guided Tissue Regeneration (GTR) which amends it with the modern concept of biological GTR. Biomechanical GTR proposed herein using amniotic membrane not only maintains the structural and anatomical configuration of regenerated tissues but also contributes to the enhancement of healing through reduction of postoperative scarring and subsequent loss of function, providing a rich source of stem cells. Amniotic membrane enhances gingival wound healing properties and reduces scarring. Excellent revascularization of the amniotic membrane is another favorable property. Amniotic membrane is potentially a good grafting material with very good wound coverage. It enhances wound healing process, good postoperative function, and esthetics without any complications. HAM could be one of the considered options in the reconstruction of oral cavity defects because it ensures good reconstruction, postoperative function, and esthetics.

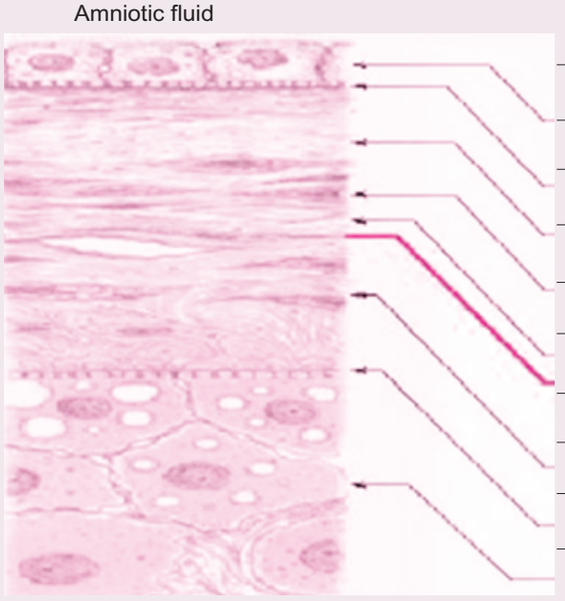
Search Strategy

Available studies and abstracts were identified through PubMed and Medline data bases (From 1910-2018) and Cochrana data bases. Key Search topic were "Human Amnion Membrane : The Magic Membrane" and relevant articles. The reference list of review article were also searched. The search term were following key words used in verious combination : Amnion membrane; Tissue engineering; Dentistry.

Discussion

Anatomy and Histology of the Amniotic Membrane

Amniotic membranes develop from extra embryonic tissue and consist of a fetal component (The chorionic plate) and a maternal component (The decidua's)



Layer	Extracellular-Matrix Composition
Amnion	
Epithelium	
Baaement membrana	Collagen types III, IV, V, laminin, fibronactin, nidogan
Compact layer	Collagen types I, III, V, VI, fibronectin
Fibroblast layer	Collagon types I, III, VI, nidogen, laminin, fibroncetin
Intermediate (spongy) layer	Collagen types I, III, IV, proteoglcens
Chorion	
Raticular layer	Collagen types I, III, IV, V, VI, proteoglycans
Basement mambrane	Collagen typa IV, fibronactin laminin
Trophoblasts	

Figure 2 : Schematic presentation of the structure of the fetal membrane at term. The extracellular matrix components of each layer are shown. Adaptedfrom Hasan N et al. (2008)

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Scientific Basis of Clinical Application

Amniotic membrane is a gift of nature which not only protects the fetus inside the womb but also has several medicinal properties. It serves as a natural barricade to protect the fetus from bacterial infection and trauma[9]. Amniotic membrane acts as a scaffold for proliferation and differentiation due to the presence of fibronectin, elastin, nidogen, collagen types I, III, IV, V and VI, elastin and hyaluronic acid[10]. Another important advantage of using amniotic membrane in allotransplant or xeno-transplant is lack of immunogenicity. Promotion of epithelialization, anti-inflammatory properties, anti fibrotic properties, antibacterial properties, and anti-angiogenic properties are confirmed by the presence of several related factors that makes amniotic membrane an ideal therapeutic for burns and wound healing. Amniotic membrane is known to promote epithelial cell migration, adhesion and differentiation and is also an ideal substrate for supporting the growth of epithelial progenitor cells by prolonging their lifespan. Finally, amnion has been also used as an allograft in general surgery for reconstructions, as an auto graft in neonatal reconstruction surgery, and as a scaffold in tissue engineering research[11-17].

Molecular Properties of the Amniotic Membrane

The human amniotic membrane at term has a number of properties that has made its clinical use a success, which includes the absence of inducing an immune reaction and having an anti-inflammatory effect. Its stromal matrix also shows a marked suppression of pro-inflammatory cytokines, IL-1α, and IL-1β expression[18].

Amniotic membrane has also been known to have natural inhibitors of MMPsand hyaluronic acid, which is a higher molecular-weight glycosaminoglycan and acts as a ligand for CD44 to the amniotic membrane stroma[19,20]. The amnion has been described as anti-angiogenetic and bacteriostatic, as well as having analgesic properties. It promotes re-epithelization and prevents scarring and functions as an evaporation barrier[21,22].

The human amnion possesses low or no immunogenicity. Cells from the fetal membranes having immune-modulatory properties may be involved in the maintenance of feto-maternal tolerance[23]. It has been shown that the Human Leukocyte Antigens (HLA) class I are expressed in amniotic epithelial and mesenchymal cells whereas HLA class II antigens are not synthesized in the cells of the amniotic membrane[24]. Epithelial and mesenchymal amniotic cells secrete a number of anti-inflammatory proteins such as Activin A, IL-1 receptor antagonist (IL-1ra), and IL-10, which are deposited within the amniotic membrane stroma[25,26].

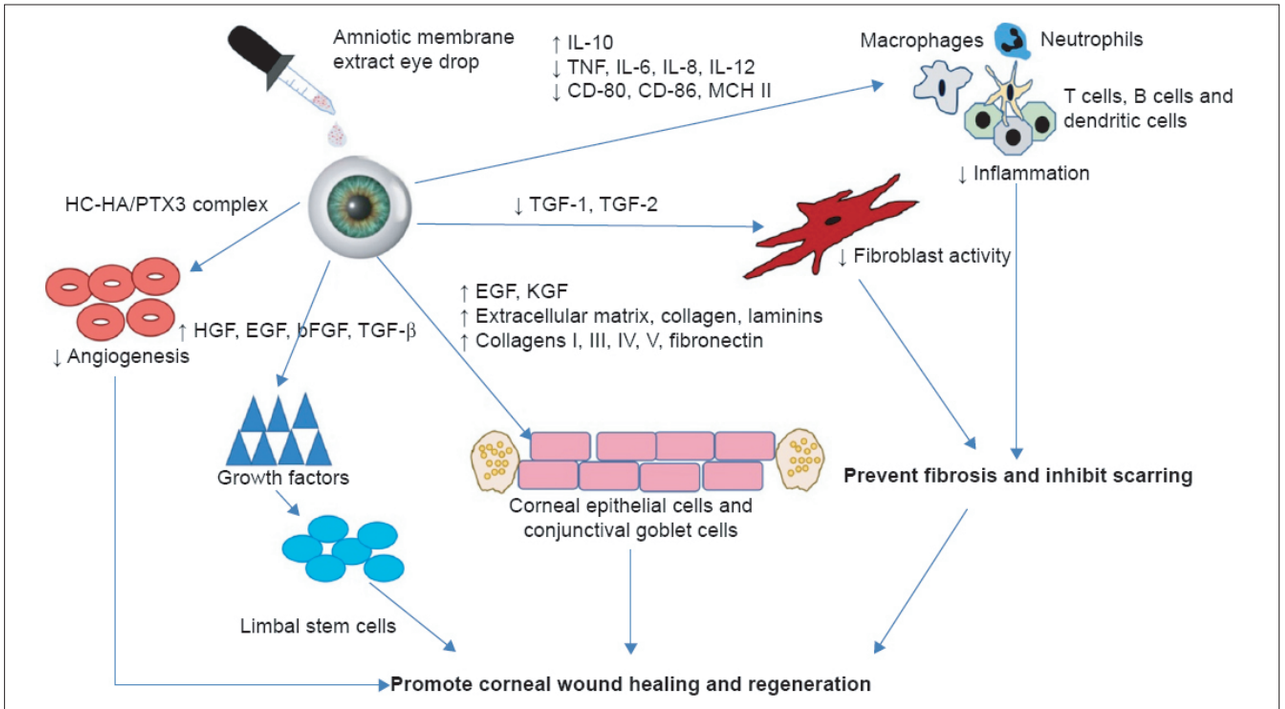

Figure 1 Proposed mechanism of AMEED role in promoting wound healing.
Abbreviations: AMEED, amniotic membrane extract eye drops; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; HGF, hepatocyte growth factor; IL, interleukin; KGF, keratinocyte growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor.

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Suppression of proteinase and MMP activation by amniotic membrane leads to decreased infiltration of inflammatory cells. Moreover, pro-apoptotic activity of the amniotic membrane has also been reported – amnion can promote the apoptosis of leucocytes[27]. Amniotic epithelial cells express the apoptosis-inducing genes Fas L, TNF, and TRAIL. Human amniotic membrane has been shown to be equally effective as autologous skin grafts, but superior to allo and xenogenic skin grafts for decreasing bacterial counts in open granulating rat wounds[28]. Kanyshkova et al. reported the presence of the antibacterial protein lactoferrin in the membrane[29].

Amniotic membrane is one of the very few human tissues that are completely avascular, hence, its ascribed anti-genic properties. Anti-angiogenic factors (Endostatin, TSP-1 and TIMPs) are produced within the amnion, however, angiogenic factors such as VEGF and bFGF have been also shown to be present in amniotic membrane[30]. Depending on the setting of in vitro and in vivo experiments with amnion or amnion-derived cells, either suppressionor promotion of neovascularization has been reported[31,32].

Amniotic membrane tissue has antimicrobial activity. Amniotic tissue produces β-defensins, which is a major group of antimicrobial peptides that are expressed by epithelial cells and form an integral part of the immune system. They protect epithelial surfaces from microbial colonization. Amniotic tissue also produces Secretory Leukocyte Proteinase Inhibitor (SLPI) and elafin. In addition to their anti-inflammatory properties, elafin and SLPI both have antimicrobial actions and act as components of the immune system to provide protection from infection. Amniotic membrane treatment with both the lactoferrin and interleukin-I receptor antagonist make the amniotic membrane antimicrobial as well as anti-inflammatory. Lactoferrin, a global multifunctional protein, has both antimicrobial as well as anti-inflammatory effects, which serves as an antioxidant and iron chelator in tissue. It is also known to suppress the production of IL-6 in the amniotic fluid during amniotic infection.

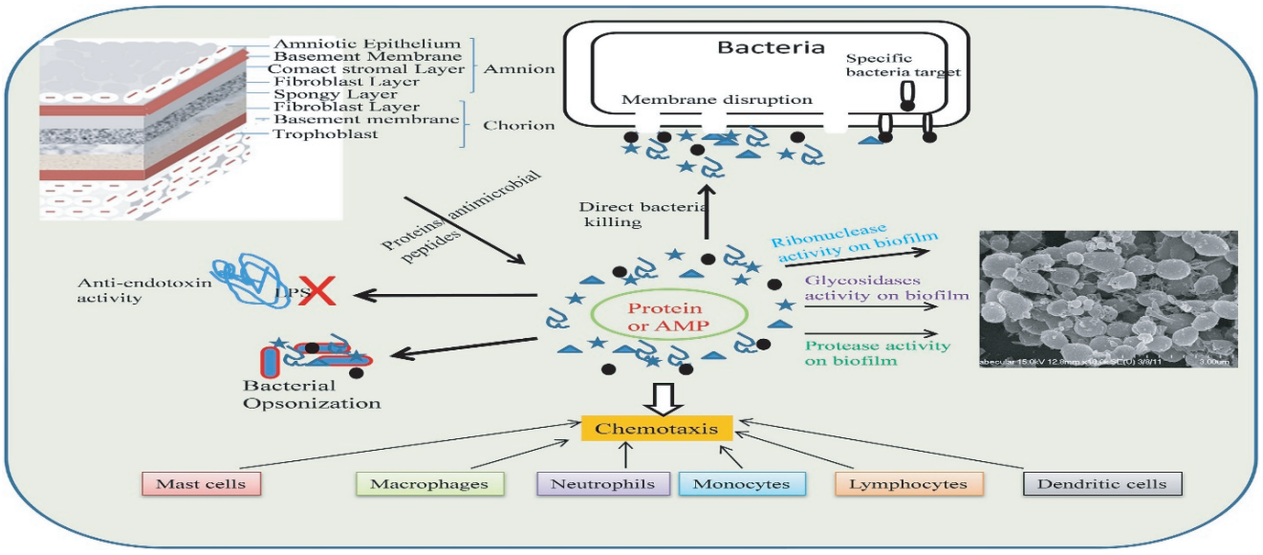
Mechanical Properties

Differentiation of some progenitor cells depends on mechanical stimulus/signals, therefore, a scaffold must create an adequately stiff environment throughout the site where new tissue is desired. Increase in stiffness enhances the stability of scaffold and prevents displacement that leads to uninterrupted healing as well as feasibility in exchange of metabolic products of involving cells during the early phase of healing. Scaffold should also have sufficient elasticity for maintaining the shear stresses of surrounding tissue. Collagen and elastin in extracellular matrix provide stiffness and elasticity for amniotic membrane, respectively. The mechanical response of amniotic membrane is time dependent that is termed as viscoelastic in nature.

Amniotic Membrane: Preparation, Processing and Preservation

Preparation

Fresh membrane is obtained from the placenta at the time of delivery, either vaginal or caesarian section. Robson and Krizekl rinsed the membrane in a 0.025% solution of sodium hypochlorite and stored at 4°C in sterile solution containing penicillin. They showed



that membranes remained sterile up to 6 weeks. Dinno et al. performed cultures to study the sterilization of amniotic membranes. Preservation with 1:40 dilution of sodium hypochlorite revealed no positive cultures until 30 days.

Processing: For Clinical Use, Amniotic Membrane Can be Prepared in the Following Forms[33]:

- Fresh membrane
- Dried membrane
- Frozen membrane
- Freeze derived irradiated membrane
- Stabilized amniotic membrane
- Cryopreserved membrane.

Preservation

Glycerol has been used as a cryo-protective agent for a long time. Because of its high osmotic pressure, it extracts interstitial water from the amniotic membrane. In this method, 80% glycerol is used for drying the amniotic membrane, which can thereafter be preserved at 4°C for a long time, although it loses some of its biologic properties. This type of preserved amnion is used for dressing burnwounds[34-44].

Preparation of AME (Amniotic Membrane Extract) and AMEED (Amniotic Membrane Extract Eye Drop) there are various methods for the preparation of AME and AMEED, without consistent standardization. One method for the preparation of AMEED consists of washing isolated human AM with normal saline (Containing 5% penicillin and streptomycin) slicing into small pieces (With a scalpel blade) and submerging in liquid nitrogen. The mixture is homogenized, centrifuged and the supernatant is collect centrifuged again and sterilized by passage through a 0.25 mm filter (Millipore, Billerica, MA, USA).17 Other methods involve pulverization, micronization or morselization of cryopreserved or dehydrated AM.

The dehydrated amnion/chorion membrane allograft can also be micronized, which allows it to be administered as a topical powder or mixed with saline to create an injectable solution or a topical gel. Use of amniotic membrane has recently increased clinically as an allograft material for chronic and acute wound care management, for scar tissue reduction, as a barrier membrane, and as a soft tissue regeneration graft. Amniotic membrane is highly useful and effective as a culture substrate[45].

Stem Cell Characteristics of Amnion-Derived Cells

Stem cell therapy is emerging as a powerful tool to generate biological substitutes and regenerate for damaged tissue with high proliferribility, differentiability, and function. The incorporation of these cells in the periodontal wound may, therefore, accelerate periodontal healing. Many efforts are under way to develop novel bioengineered wound-healing products, including involvement of Mesenchymal Stromal Cells (MSCs) in the wound-healing process.

At present, tissue engineering uses Human Embryonic Stem Cells (HESCs) as allogenic cells. The HESC lines have been derived from the inner cell mass of blastocyts that are 3–5 days old, originally described by Thomson et al the ongoing researches have suggested that the amniotic epithelium retains the reservoir of stem cells throughout the pregnancy as the researchers have successfully generated ectodermal, endodermal and mesodermal cell lineages using HESCs[46-48].

The current understanding of pluri-potency is based on extensive studies of mouse and HESCs, and more recently also induced pluripotent stem (iPS) cells[49,50]. In 2004, Tamagawa et al. reported the isolation of a pluripotent stem cell line derived from cultured whole HAM. These stem cells contributed to the formation of chimeric mouse/human embryoid bodies in vitro, giving rise to cells with characteristics of the primordial liver, lung, and digestive tract but also to neural, epithelial, and hematopoietic cells, and blood vessels. Human amnion-derived cells seem to give rise to cells of all three germ layers[51].

Amniotic Membrane for Potential Use in Tissue Engineering

An important component of tissue engineering is the supporting matrix upon which cells and tissues grow, also known as the scaffold. Scaffolds must easily integrate with host tissue,and provide an excellent environment for cell growth and differentiation. Most scaffold materials are naturally derived from mammalian tissues. The amniotic membrane is considered an important potential source for scaffolding material.

Amniotic membrane has biocompatibility, low immunogenicity, adequate mechanical properties (Permeability, stability, elasticity, flexibility, resorbability) good cell adhesion, and easy delivery of bio-modulatory agents such as growth factors and genetic materials. The attachment of a cell to a scaffold is largely affected by the components of the scaffold's extracellular matrix. The presence or absence of certain extracellular matrix molecules such as collagen, laminin, fibronectin, and vitronectin within any basement membrane has a huge influence on the adhesion and growth of the overlying stem cells. In addition to allowing the cells to attach and migrate, the extracelllar matrix molecules also serve as adhesion ligands,

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which transmit signals via their interaction at cell surface receptors. When epithelial and mesenchymal cells are seeded on a cellular scaffold created from the amniotic membrane, the cells were highly interconnected and capable of penetrating the porous structure of the amnion scaffold. Cultivation and seeding of epithelial cells on an amnion scaffold is a frequently used method for ocular surface and skin reconstruction[30-32]. And finally, cultivation of endothelial cells on an amniotic membrane scaffold has also been reported as a potential approach for vascular TE.

Biocompatibility is said to be the property of being biologically compatible by not producing a toxic, injurious, carcinogenic, or immunological response in living tissue, and is also a major prerequisite for choosing a scaffold[52-53]. In addition, their mechanical properties should include permeability, stability, elasticity, flexibility, plasticity, and resorbability at a rate congruent with tissue replacement[54]. Scaffolds should also allow cell adhesion and the potential for delivery of biomodulatory agents such as growth factors and genetic materials[55-57].

Historical Use of Placental Allografts

The first recorded clinical use of amnion tissue was for use in skin transplantation in 1910[1]. Shortly thereafter, it was frequently used to treat ulcerated skin conditions, reconstruction of vaginal malformed organs,and vestibuloplasty[58-60].

The clinical use of cryopreserved amnion allograft in ophthalmic surgery was first reported in 1997[61]. Today these allografts are commonly used in ophthalmic surgery, and literature suggests that both cryopreserved amnion allograftand dehydrated amnion allograftprovide results equivalent to conjunctive auto graft tissue[62,63]. It has been used as an adhesion barrier in spine and orthopedic procedures as well as in the treatment of chronic wounds.

Clinical Applications of Human Amnion

Human amnion has a long history of clinical applications. It was reported for the first time as a biological dressing to heal skin wounds a century ago. In the management of open wounds, the major goal is to obtain a clean and closed wound in the shortest time possible, thereby preventing fluid, heat and nutrient loss as well as wound infection, pain, and decreased mobility. Amniotic membranes are efficiently used as allografts for treating skin burns, open and non-healing ulcers; pressure sores and surgical, infected, and traumatic wounds[64,65]. An alternative treatment to manage wounds in the oral cavity, such as the tongue, buccal mucosa, vestibule, palatal mucosa and floor of the mouth; in the reconstruction of the oral cavity, bladder, and vagina; tympanoplasty, arthroplasty, and so forth. Its adhesive and tight contact with the injured surface promotes hemostasis and good pain relief due to exposition of nerve fibres. Good biocompatibility and mechanical properties such as permeability, stability, elasticity, flexibility, plasticity, and resorbability also make it a promising scaffolding material in tissue engineering as in cell adhesion, and the potential for delivery of biomodulatory agents such as growth factors and genetic materials. Anti-inflammatory and antiscarring property of amniotic membrane have shown decreased necrosis and rapid healing of ulcers with Herpes Simplex Virus (HSV) varicella zoster virus infected tissues, erythema multiforme major (Stevens–Johnson syndrome), and cervical necrotizing fasciitis. HAM has been tried in the reconstruction of temporomandibular joint ankylosis because it prevents fibrosis and reankylosis when used as an interpositional material. Amniotic membrane is even used as a carrier for local delivery of various drugs such as antibiotic netilmycin (NTM) and antiviral drugs such as acyclovir (ACV) and trifluridine (TFU). Amnion has been tried as a graft material after vestibuloplasty where it prevents secondary contraction after surgery and maintains postoperative vestibular depth[66-77].

Current Clinical Uses of Amniotic Tissue in Dentistry and Oral Surgery

Amniotic membranes have already been used extensively as biologic dressings on different part of body. The laminin structure of amnion tissue is nearly identical to that of native human tissue such as oral mucosa. Reconstruction of a buccal mucosal defect after excision of speckled leukoplakia using HAM has been reported with a promising result[78].

Tooth socket preservation has become a key component of contemporary clinical dentistry with amniotic membrane and bone particulate have been utilized for socket augmentation[79]. Many studies have been found effective using a combination of membrane and bone graft in patients with serious periodontal defects or patients requiring bone augmentation for implant surgery[80,81]. It also prevents the osteogenic potential thereof and causes the repair of bone defects, thus amniotic membrane has a positive effect on the guided bone regeneration process[82]. Moreover, human amnion membranes have recently been reported as a suitable platform in facilitating osteogenic differentiation for both stem cells and apical papilla cells[83,84]. When covering over the defects on maxillary and

mandibular bone, the acellular human amnion membranes were found to promote injury-healing process while improving bone induction[85]. A novel allograft composed of amnion tissue has recently been introduced for periodontal plastic surgery. Forroot coverage, increased tissue thickness, and increased attached gingival tissue by guided tissue regeneration[86,87].

The ability of processed dehydrated allograft amnion to self-adhere eliminates the need for sutures. The procedure becomes less technically demanding reducing the surgical time. The ability to self-adhere makes processed dehydrated allograft amnion an attractive option for multi-teeth procedures and recession defects in particularly posterior region. Processed dehydrated allograft amnion may provide an effective alternative to auto graft tissue in the treatment of shallow-to-moderate Miller Class I and II recession defects.

The clinical usefulness of the hyper-dry amniotic membrane as an intraoral wound-dressing material has been studied, and the results suggest that the hyper-dry amniotic membrane is biologically acceptable to oral wounds and could be a suitable clinical alternative for the repair of the oral mucosa[88]. A successful closure of oro-nasal fistulas was observed in minipigs using interposed grafts of cryopreserved HAM, offering a simple and effective technique for tension-free closure of such fistulas[89, 79-85]. Amniotic membrane is also used in vestibuloplsty and as an interpositional materials for TMJ gap arthoplasty[90].

The Future of Amniotic Tissuein Dentistry

Amniotic membrane hydrogel, amniotic membrane powder or amniotic membrane extract may use in dentistry for the treatment of periodontal pocket andamniotic fluid or steam cell in oral sub mucous fibrosis as inter-lesional injection and in conjunction with bone particulate for bone defect after cyst enucleation or socket preservation after extraction for dental prosthesis.

Conclusion

The safety, logistical, and surgical advantages of amnion membrane are vast. Dental applications of amniotic membrane are currently showing great promise in various specialties of dentistry. Amniotic membranes have already been used extensively in medical field as biologic dressings in ophthalmic, abdominal, and plastic surgery. Amniotic membranes have a rich inheritance of collagen types I, IV, V, and VI, proteoglycans, laminin and fibronectin. Collagen is well tolerated and bio absorbable, has hemostatic properties and encourages migration of adjacent autogenous connective tissue and epithelial cells over its surface. Laminins exhibit a variety of biological activities including promotion of cell attachment, growth, and differentiation of number of cell types. Fibronectin is involved in many cellular processes including tissue repair, blood clotting, cell migration, and adhesion. The use of this novel biological membrane is rising in various fields of tissue engineering, medicine, regeneration biology and stem cell research. The clinical application of amniotic membrane not only maintains the structural and anatomical configuration of regenerated tissues, but also contributes to the enhancement of healing through reduction of postoperative scarring and subsequent loss of function, providing a rich source of stem cells. Other properties of the AM include anti inflammation, ant fibrosis ant scarring, antimicrobial, low immunogenicity and reasonable mechanical property, which are all important for use in tissue engineering. However, further research and long-term clinical trials investigating the full potential of this stem cell reservoir are still warranted to strengthen the fact that amniotic membrane is indeed a reservoir for regeneration.

Disclosure

All the authors declared no competing interests.

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Case Report

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Implant in Posterior Maxilla and Subantral Augmentation: A Case Report

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Abstract

Partial or complete edentulous posterior maxilla is one of the most common condition in dentistry. Many unique and challenging condition are presented in implant dentistry. The surgical procedure is to increase the bone height and width to create a bed for implant. Grafting of the maxillary sinus to overcome the problem to reduce vertical available bone. The maxilla has the thinner cortical plate on the facial skeleton in comparing to mandible. Loss of posterior maxillary dentition results in decrease of bone height and width. The article describes an advanced clinical procedure for augmentation of subantral space with allograft and placement of implant fixer simultaneously.

Key words

Implant; Maxilla; Antrum; Augmentation; Allograft.

Introduction

The maxilla has the thinner cortical plate on the facial skeleton in comparing to mandible. In addition, posterior maxilla has a finer trabecular bone than the other dentate region[1]. Loss of posterior maxillary dentition results in decrease of bone width as well as height[2]. The posterior maxilla continues to remodel towards midline as the bone resorptioncontinues[3].
Before posterior implants are considered a minimum of a healthy natural canine tooth or an implant abutment in the canine region is required.

Abnormal intraoral conditions like

- Inadequate oral hygiene
- Untreated periodontal disease of the residual dentition
- Severe malocclusion
- Active infection.

Other conditions like

- Decreased crown height space
- Poor bone density
- Implant size.

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May compromise the final outcome of sinus grafting.

Radiographic Evaluation

Waters Projection

The most common medical plain film radiograph used for evaluation of the maxillary sinus in the occipitomental projection, also termed as water's projection the film is taken with the patient's head is tilted upwards approximately 40 degrees, allowing a clear evaluation of superior, lateral and medial aspects of maxillary sinus. Waters projection is often complimented with similar plain films such as CaldWell, lateral view and submentovertical.
Water's projection radiograph has little use for diagnosis and treatment planning in implant dentistry.

Panoramic Radiograph

It is often uses as a pleminary diagnostic radiograph in implant dentistry. This radiograph can direct visualization of the anterior, lateral, and inferior regions of the maxillary sinus, Available height can place the patient category of SA 1 and SA 4. The SA 2 and SA 3 are less obvious, because the height range from 5 to 10 mm of bone may be influenced by film magnification.

Periapical Radiograph

In these radiograph the roots of the maxillary teeth may appear to project directly into the sinus and may produce conical elevations on the floor of the sinus,yet there is always a layer of bone and mucosa covering these roots.
It has a little value in evaluation of the sinus in the posterior maxilla.
Cone Beam Computerized Tomography (CBCT) is an effective diagnostic modality. It is important as it provides 3D Image with idea on

- Sinus membrane
- Ostiomeatal Complex

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- Anatomical Variations
- Bone density and
- Virtual implant placement

Considerations specific to sinus grafts
- Smoking
- Chronic maxillary Rhino sinusitis.

Clinical Assessment

The surgical procedure is to increase the bone height and width to create a bed for implant. Grafting of the maxillary sinus to overcome the problem.

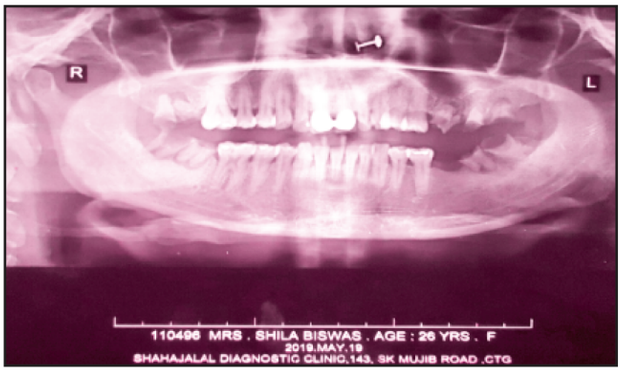
- The first Sub-Antral treatment option, SA-1, when sufficient bone height is available to permit the placement of endosteal implants following a usual surgical protocol.
- The SA-2, is selected when 10 to 12 mm of vertical bone is present. Osteotomy is done by crestal approach. A slow elevation of the sinus floor 0.5- to 1.0-mm increments up to 2mm. Sometimes bone graft materials is placed in the osteotomy site.
- The third approach SA-3, is indicated when at least 5 mm of vertical bone and sufficient width are present. The lateral maxillary wall approach is performed. Autogenous bone, alloplast, and/or allograft material is placed. An onlay graft in conjunction with the sinus augmentation is recommended when ridge width is Division B or C.
- In the fourth option SA-4, Antrum is first augmented. This option is indicated when less than 5 mm remains between the residual crest of bone and the floor of the maxillary sinus.

Case Report

Mrs. "S" 26 year's old lady presented with the complaint of missing posterior teeth and difficulty in chewing foods. She was relatively all right five years back then she had toothache then she went to local doctor. Therefore she took some medicine. The patient discontinued to go to the doctor and gradually her teeth became more carious and it was broken to some pieces. Now she wants to replace the broken teeth and restore the chewing function.

The patient is non-diabetic, non- hypertensive and not associated with any illness.

On clinical intraoral examination multiple broken down roots were found on the molar region both in maxilla and mandible. Her oral hygiene was average. Therefore she was advised for routine OPG. A diagnostic cast was prepared.



Diagnostic Orthopantomogram

She was diagnosed as a case of Kennedy Class –II, edentulous arch.

Her treatment plan was
Stage 1: Extraction of the broken roots and socket graft.

Stage 2: Sinus Augmentation (SA-3) by direct window osteotomy approach as well as implant placement and progressive loading.

Procedure

Premedications
- Local Anesthetic medications
- Oral antimicrobial rinse
- Glucocorticoid medications
- Decongestant medications
- Allergic medications
- Cryotherapy.

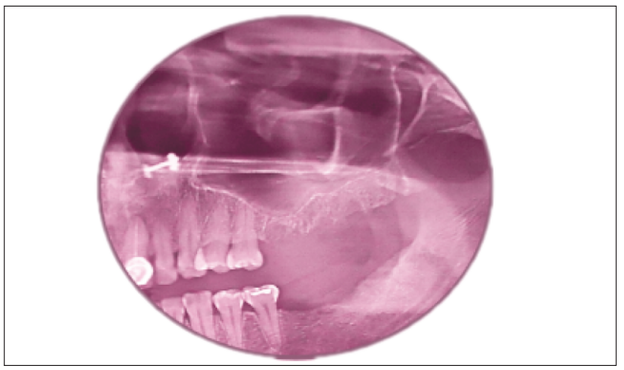
Surgical Technique

First Stage

With all asepsis and precautions her all the broken down roots were removed and the extracted socket graft was done with alloplastic materials in the maxillary region.

Second Stage

After six month the second surgery was performed. With all asepsis and precautions following steps were taken.



Radiographic evaluation 6 months after 1st stage

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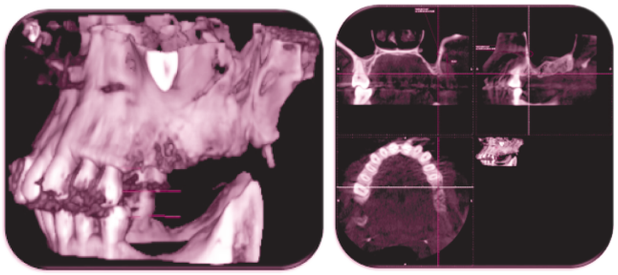
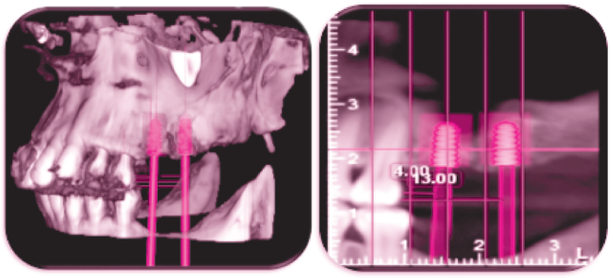
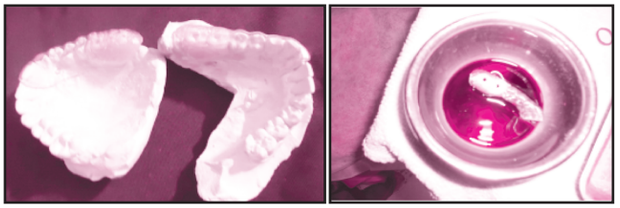


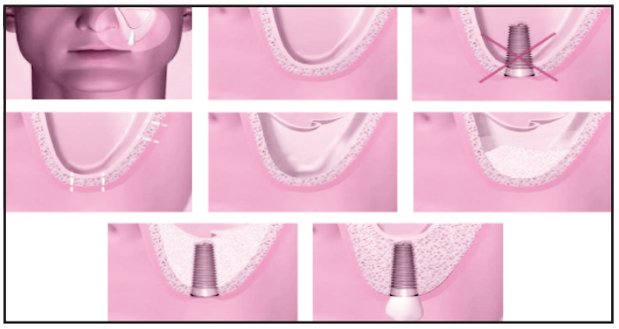
Computerized tomography (CBCT)



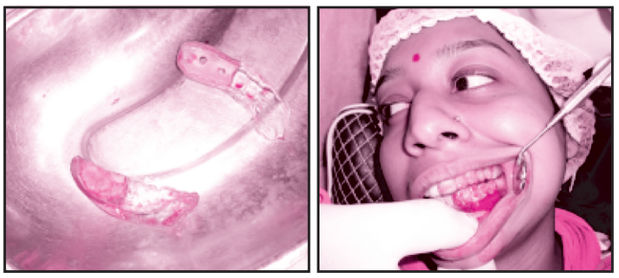
CBCT Analysis



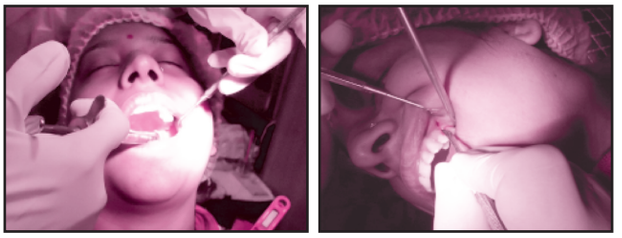
Surgical Templates



Over view of implant placement in posterior maxilla



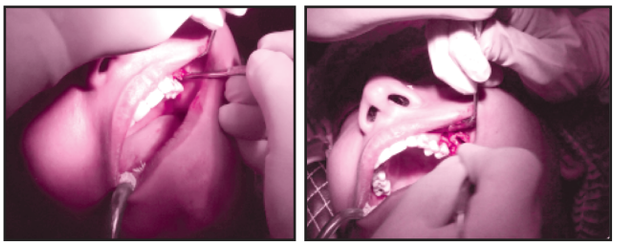
Surgical Templates
The Templates were used to assist the proper placement Prosthetic guided implants.



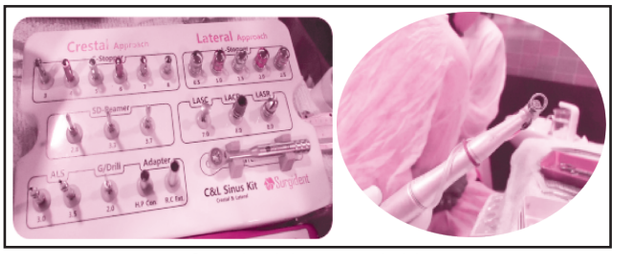
Administration of Local Anesthesia (V2 block)

Incision

Surgical Procedure

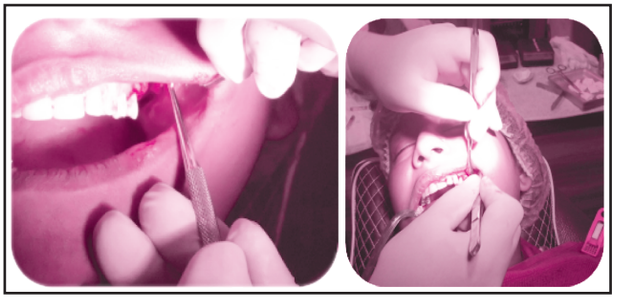


Full-thickness buccal flap was reflected with 4 mm clearance



Surgical Approach kit

Lateral window approach



Elevation of the sinus membrane with specially designed lifters

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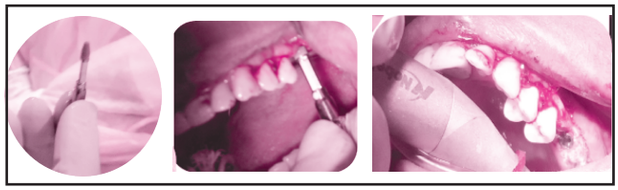
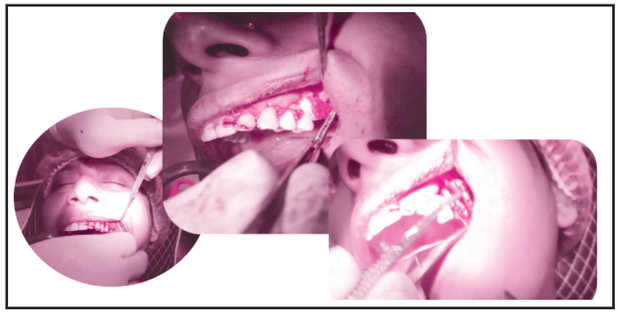
Implant Osteotomy was performed with standard protocol

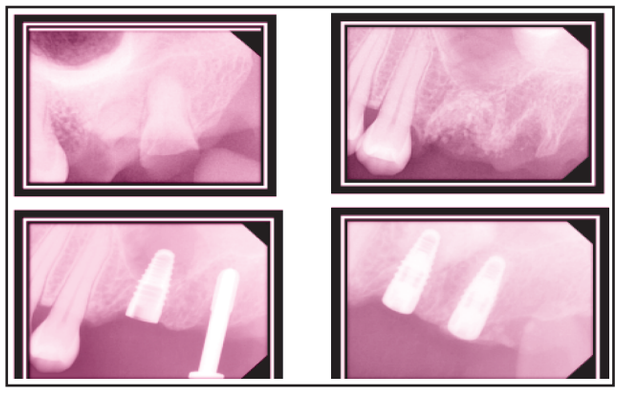


Endosteal Implant placement was placed

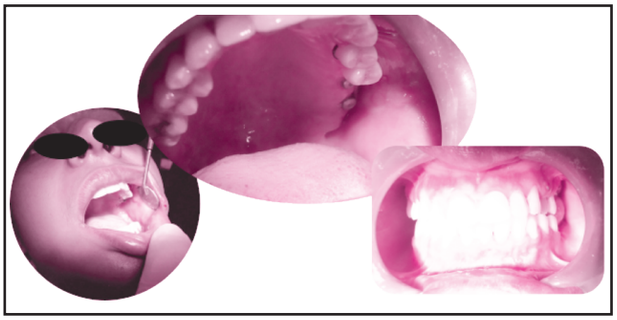
Bone graft materials was placed



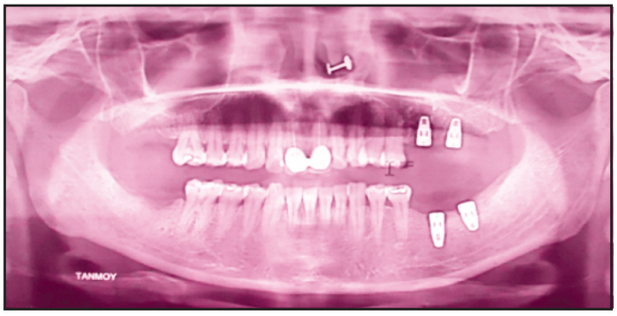
Absorbable membrane was placed and wound was closed



Series of operative RVG



Post surgical follow up after one month



Post Surgical Orthopentamogram after one month

Discussion

Conventional strategies for a sinus floor elevation involve a variety of methods and medical instruments.Vast knowledge and skills are required for this technique. The opening of a bony window in the lateral wall of the maxillary sinus can be performed by the traditional method presented by Tatum in 1977 and published by Boyne and James in 1980[4,5.] The crestal bone height can be elevated by the grafting of the maxillary sinus with intraorally harvested autogenous bone or other grafting material[6].

A lack of visual control of the site characterizes almost all of the transalveolar techniques. Therefore, while performing an osteotomy, a surgeon must be experienced in detecting the changes on the bone quality and the resistance to penetration during the osteotomy. The use of piezoelectric instruments to reach the sinus floor does not consume more time than conventional surgical techniques. These instruments are safe and involve less risk of membrane perforation after a “learning curve” in handling the instruments; however, they are expensive and training dependent[7,8]. So, the complementary use of digital periapical radiographs is suitable to control osteotomy progress. The main challenge during a maxillary sinus floor elevation is to maintain the integrity of the sinus membrane[9]. Membrane perforation is considered one of the biggest intrasurgical complications and can lead to a postoperative sinus infection, which is the principal cause of failure[9].

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Therefore, in the presented technique, the first precaution taken to preserve the integrity of the sinus membrane involved the strategy implemented to perform an osteotomy. The lateral approach kit was used with specially designed safe ended lifting instruments. Aimetti et al affirm that the eventual lacerations of a sinus membrane do not cause significant onsequences or implicate complications in healthy membranes[10.11]. No sonusmembrane was perforated since it has been corroborated by the Valsalva maneuver[12].

Del Fabbro et al have suggested that only the elevation of the Sinus membrane may be sufficient to obtain new bone formation in the newly created space between it and the sinus floor, so the placement of the grafting material has been questioned[13]. However, the time between the Sinus membrane detachment and the complete bone formation is large, and it does not allow immediate implant placement. Thus, another important factor for a successful surgery involves the correct detachment of the sinus membrane from the subjacent sinus floor bone and the graft material placement. Several surgical procedures in which special instruments are used have been introduced (e.g the use of balloon catheter piezoelectric instruments hydraulic or gel pressure[14-19]. These procedures involve diverse complications already described and their use depends on the acquisition of an expensive surgery sinus lift kit. In the presented technique, the instrument used for detaching the membrane was a special design, which has a rounded non-sharp edge. The instrument used for the bone substitute delivery was a specially adapted instrument based on the “piston” operating principle.

Conclusion

In the present technique lateral window approach was applied to access the sinus and, principally to protect the Sinus membrane; however, experienced-surgeons are required. A bone substitute was placed using a specially adapted instruments, which allowed for comfortable work and precise control on the amount of used material. In all of the cases, a satisfactory augmentation and bone volume were achieved, allowing immediate implant placement.Indication of this technique with immediate implant placement refers to situations of moderate bone reabsorption. The use of a bone substitute should not be excessive.This technique overcomes the classical drawbacks in favor of sinus membrane preservation. The present preliminary study requires a greater number of cases. More patients, along with long-term controls and evaluations would help to better determine success of the presented technique.

Disclosure

All the authors declared no competing interest.

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