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RESEARCH ARTICLE

A COMPARATIVE EVALUATION OF THE EFFICACY OF CHORION MEMBRANE WITH AND WITHOUT 2% METRONIDAZOLE GEL IN PERIODONTAL POCKET THERAPY

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ABSTRACT

Background: The aim of this study is to comparatively evaluate the efficacy of dehydrated human chorion membrane with and without 2% Metronidazole gel in periodontal pocket therapy in patients with moderate to severe periodontitis. **Methods:** 40 patients were recruited for the study and were divided into 2 groups of 20 patients each. The clinical parameters assessed were plaque index, gingival index, pocket probing depth and clinical attachment loss. The clinical indices were assessed at baseline, 1 month and 3 months and pocket probing depth and clinical attachment loss were assessed at baseline and 3 months postoperatively. Surgical therapy involved open flap debridement for both groups followed by placement of chorion membrane in Group 1 and placement of chorion membrane with application of 2% metronidazole gel on the flap side of the membrane in Group 2.
Results: For all the clinical parameters assessed, highly significant ($p<0.01$) reduction was observed with respect to the plaque and gingival index scores within the group. Intergroup comparison, however, showed no significant difference. Pocket probing depth and clinical attachment loss showed highly significant ($p<0.01$) improvement from baseline to 3 months within the groups as well as between the two groups. However, Group 2 (chorion membrane with 2% metronidazole gel) showed a larger effect size than did Group 1 (chorion membrane alone). **Conclusion:** The results establish that although both treatment modalities i.e., chorion membrane alone or in conjunction with 2% metronidazole gel yielded good results, the latter proved to be more effective and can be promising for the treatment of periodontal pockets in patients with chronic periodontitis.

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INTRODUCTION

Periodontal disease is a complex multi-factorial disease characterized by the destruction of periodontal tissues and loss of connective tissue attachment. Periodontitis is the result of complex interrelationships between infectious agents like bacteria and host factors (Flemming, 1999 and Page, 1997). Periodontal diseases are considered infections of the periodontium, because there is bacterial etiology, an immune response and tissue destruction (Haffajee, 2000). While it might be impossible to strictly interpret the Koch – Henle Postulates in relation to periodontal diseases, it is for most part universally accepted that they are the result of mixed bacterial infections that require the participation of a very limited number of the members of the anaerobic microbiota inhabiting the subgingival region and results in the destruction of supporting structures of the teeth (Haffajee, 2000 and Darveau, 2000). It has been long recognized that bacteria are primary ecological agents of periodontal diseases (Loe, 1965). Destructive periodontal disease is associated with a variety of microbial species, including the major pathogens Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis and Bacteroides forsythus and some putative pathogens including Prevotella intermedia/nigrescens, Campylobacter rectus, Fusobacterium nucleatum, Peptostreptococcus micros, Eubacterium species, β- haemolytic streptococci, various gram-negative enteric rods, pseudomonas, enterococci, staphylococci and yeasts. These putative pathogens associated with periodontal diseases are susceptible to a variety of antiseptics and antimicrobials (Haffajee, 2000; Loe, 1965; Moore, 2000; Haffajee, 2000). Periodontal disease initiation and progression occurs as a consequence of the host immune inflammatory response to oral pathogens. Periodontal pathogens produce harmful by-products and enzymes (e.g hyaluronidases, collagenases, proteases) that breakdown

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extracellular matrices, such as collagen, as well as host cell membranes, in order to produce nutrients for their growth and possibly subsequent tissue invasion. Many of the microbial surface proteins and lipopolysaccharide molecules are responsible for eliciting a host immune response, resulting in local tissue inflammation and subsequent tissue destruction.

One of the goals of periodontal therapy is to restore periodontal tissue lost through periodontal disease. The later goal prompts us to evaluate the concept of new attachment or regeneration and reattachment or repair (www.drbui.com/ artattachment.html). The objectives of periodontal therapy are to eliminate the periodontopathogens and/or the pathological changes in the pocket wall so as to create a stable and maintainable state which will promote periodontal regeneration. In the course of periodontal history, several techniques have been proposed and promoted to treat periodontal pockets and thus improve their prognosis. Shallow pockets can generally be managed by thorough scaling and root planing and adequate oral hygiene maintenance whereas, deeper pockets can sometimes prove to be a challenge due to inaccessibility and higher microbial concentration towards the base. Also, the type of bone loss may present to be a potential complication. These have been attempted to be treated with open flap debridement, guided tissue regeneration and local and systemic use of various antimicrobial agents with variable results.

According to Kornman and Robertson (Kornman, 2000), success of any periodontal treatment depends upon a number of factors:

1. Patient related variables such as patients with a high risk for systemic related reactions like diabetes, connective tissue disorders, drugs which affect the periodontium.
2. Defects and tooth factors such as tooth and bone defect morphology and the overall defect depth and associated bony walls have all been related to clinical outcomes.
3. Infections – a number of recent studies have correlated levels of infection with clinical healing responses.

Colonization of membranes by micro-organisms has been studied by a number of authors (Mombelli, 1993; De Sanctis, 1996; Yoshinari, 1998). Although there have been studies which showed gain in attachment and over all improved post-operative results following Guided Tissue Regeneration without the use of local or systemic antibiotics in the presence of microbial colonization (De Sanctis, 1995), Selvig et (1992), Nowzari and Slots (1994), Simon *et al* (1994) and Nowzari *et al* (1995) all found a correlation between higher levels of membrane contamination/ infection and reduced clinical attachment level gains (Sela, 2009). This led to concept of using systemic or local antimicrobial agents to improve the overall results of periodontal therapy. A number of different agents have been experimented with to evaluate their efficacy. One of the most popularly prescribed systemic antibiotic combination is amoxicillin and Metronidazole as it has proved to be extremely beneficial in reducing the microbial load in surgical and non-surgical periodontal treatment (Zandbergen, 2004 and Sgalastra, 2012). However, with systemic antimicrobial therapy, there is always a disadvantage of developing resistant bacterial strains and adverse side effects. Thus, localized use of antimicrobials was studied in the form of local drug delivery and antibiotic loaded guided tissue regeneration membranes. In the present study, Metronidazole was selected as it is particularly suitable for the treatment of periodontitis due to its restricted spectrum of activity against obligate anaerobes and its limited side effects, compared to those of tetracyclines (Slots, 2002 and Ghayoumi, 2001). Yu *et al* (2009) observed that metronidazole was rapidly taken up by fibroblasts which made it easy for the drug to reach its minimum inhibitory concentration in the fibroblasts. Tomas *et al* (2007), showed that 100% of the obligate anaerobes isolated from periodontitis lesions and saliva were sensitive to metronidazole, but developed resistance to other antimicrobial agents, such as amoxicillin. Metronidazole has already been proven to be effective in periodontal pocket therapy in the form of local drug delivery and loaded guided tissue regeneration membranes. Collagen membranes have been widely used for Guided Tissue Regeneration since the late 1980s. Since type I collagen is a predominant component found in the periodontal connective tissue, several commercially available collagen membranes have been developed using type I collagen as their major component (Buayaratavej, 2001). In addition, collagen materials also possess additional advantages including hemostasis, chemotaxis for periodontal fibroblast and gingival fibroblasts, weak immunogenicity, easy manipulation and ability to augment tissue thickness. Human dehydrated chorion membrane can be considered to be a third generation Guided Tissue regeneration membrane (Buayaratavej, 2014). As previously mentioned, it inherently contains collagen Type I, III, IV, V and VI and therefore possesses most of the properties and advantages of other commercially available collagen membranes (Raja, 2000). The membrane also serves as a fibrillar scaffold for vascular and tissue growth and is resorbed by the enzymatic activity of macrophages and neutrophils. In addition to the collagen, various growth factors including PDGF-AA, PDGF-BB, bFGF, TGF-β1, EGF, VEGF, and PlGF are also present in chorion membrane. These growth factors are known to play a very important role in regeneration and repair of periodontal tissues (Raja, 2009).

MATERIALS AND METHODS

Experimental design: In the current clinical study 2 different treatment modalities were employed for the treatment of periodontal pockets - by means of human placental membrane and without 2% Metronidazole gel in patients with chronic periodontitis. The patients were randomly assigned to Group 1 and Group 2. Patients belonging to Group 1 were treated with open flap debridement followed by placement of dehydrated human chorion membrane, whereas, Group 2 received chorion membrane with 2% metronidazole gel on the flap side after open flap debridement. Plaque index and gingival index scores were measured at baseline, 1 month and 3 months. Pocket probing depth and clinical attachment loss were measured at baseline and 3 months (Fig 1, 7).

Study Population: A total of 40 patients were recruited for this study (21 males and 19 females; 25 to 55 years of age). Inclusion criteria was: 25 to 55 years of age, subjects with moderate or severe periodontitis with pocket probing depth ranging from 5mm to 8mm, teeth anterior to the first molars in both, the maxillary and mandibular arch, systemically healthy patients, subjects with ability to maintain good oral hygiene compliance during Phase I therapy, vital teeth, and endodontically treated non vital teeth.

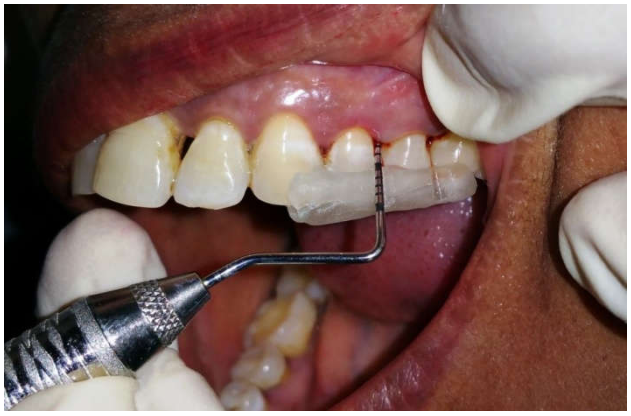

Figure 1. Pocket probing depth and clinical attachment level measurement

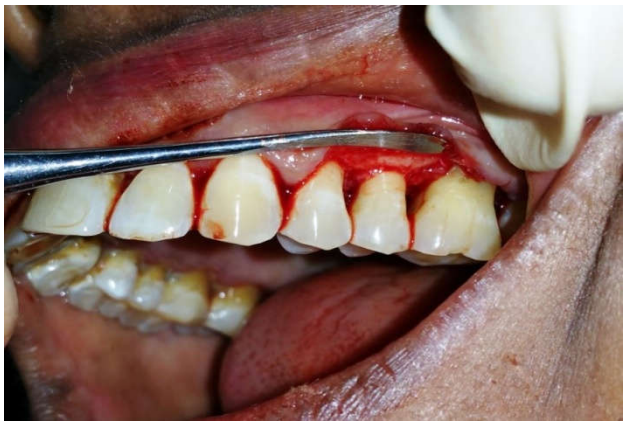

Figure 2. Flap Reflection and debridement

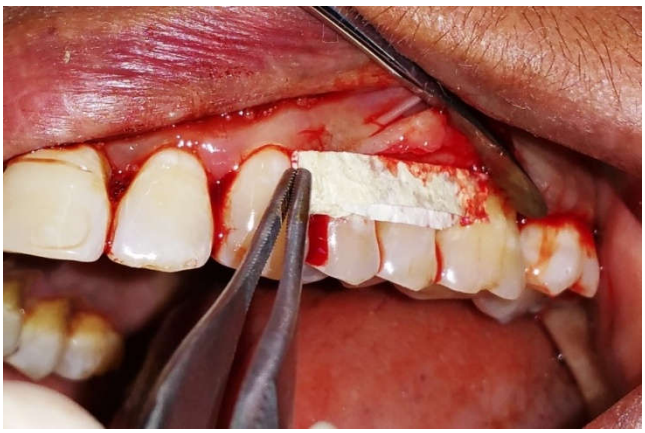

Figure 3. Application of Chorion Membrane

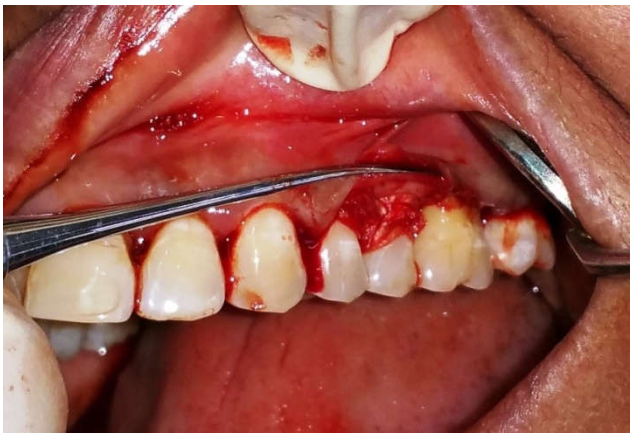

Figure 4. Chorion Membrane in Place

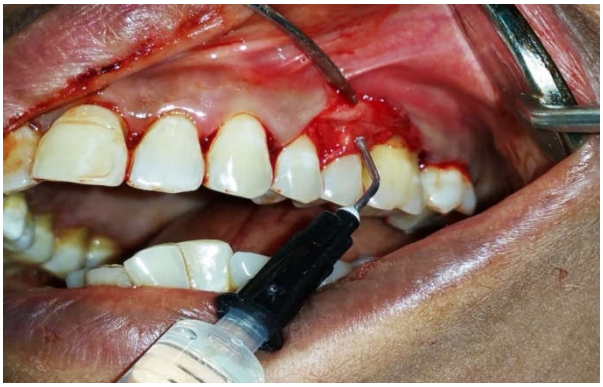

Figure 5. Application of 2% Metronidazole gel in Group B

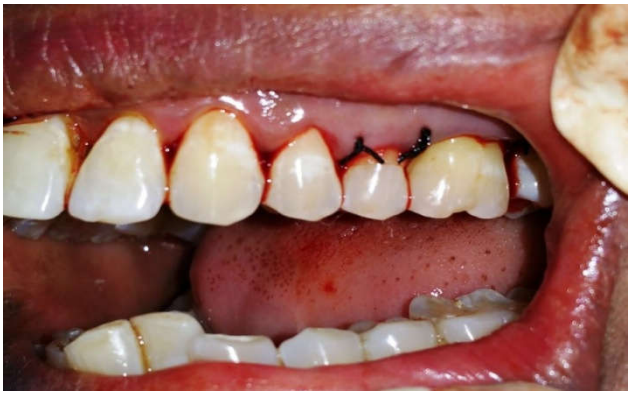

Figure 6. Interrupted sutures given with 4-0 silk sutures

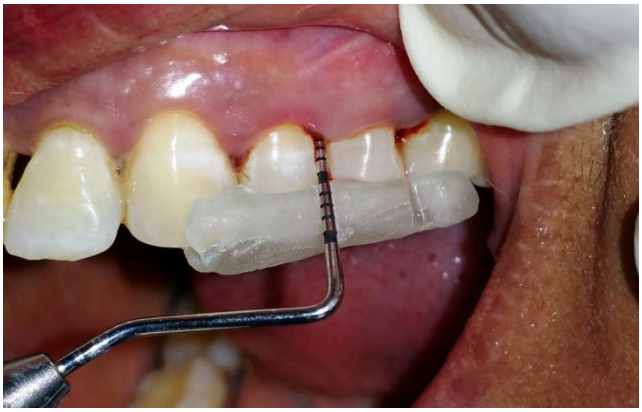

Figure 6. Pocket probing depth and clinical attachment level evaluation at 3 months follow up

Exclusion criteria included previous periodontal surgery at the site in the past 6 months, presence of traumatic bite not requiring correction by occlusal adjustment, multirooted teeth as the furcation could present as a plaque retentive area and lead to the failure of the treatment, previous regenerative surgery at the same site, teeth with poor, questionable and hopeless prognosis; teeth with presence of acute infection; tobacco consumption in any form; alcohol consumption; mobile teeth; subjects unable to maintain adequate oral hygiene; presence of periapical infection, intrabony defect, proximal caries in the tooth being treated or teeth immediately adjacent to the area of surgery; non-cooperative patients; subjects who were medically compromised or under any therapeutic regimen that would alter the outcome of the periodontal therapy; pregnant and lactating women; presence of severe cervical abrasions, erosions or root caries that would require restoration.

Materials: The dehydrated human chorion membrane and commercially available 2% Metronidazole gel was used.

Initial therapy: Each participant was made to undergo a thorough medical and dental examination and was briefed about the nature and duration of the study and informed consents were obtained. At Phase I therapy, oral prophylaxis was performed and at every subsequent visit strict oral hygiene instructions were reinforced. Thorough supragingival and subgingival scaling and root planing was done.

Surgical technique: Peri-oral preparation was done with povidone iodine, followed by rinsing of mouth with 10ml of 0.2% Chlorhexidine for 1 minute and the standardized surgical procedure was performed as follows. The surgical area was prepared and adequately anesthetized using 2% Lignocaine HCl containing 1:80000 adrenaline by infiltration anaesthesia. A full thickness muco-periosteal flap was elevated following which thorough debridement was done to remove all granulation tissue and residual calculus (Fig. 2). In test Group 1, following debridement, only Chorion membrane was placed 3 mm beyond the bone crest in an apical direction and proximally extending into the interdental region (Fig. 3, 4). The flap was then sutured in place using 4-0 silk sutures with an interrupted suturing technique (Fig. 6) . Following mechanical debridement, in Group 2, Chorion membrane was placed, extending 3mm beyond the defect area in an apical direction and proximally extending into the interdental region. Then 2% Metronidazole gel was applied on the membrane on the flap side (Fig. 5) . The flap was then approximated and sutured in place using 4.0 silk sutures with an interrupted suturing technique. Post-operative anti-inflammatory drugs were prescribed along with 0.12% Chlorhexidine mouthwash. The patients were recalled after 10 days for suture removal.

Statistical Analysis: The sample size calculation determined that 20 subjects per treatment group would provide 80% power to detect a true difference between the 2 groups using Pocket Probing depth and clinical attachment loss as the primary outcome variables. Accordingly, a sample of 20 subjects per group (40 in total) was recruited. The statistical analysis was performed using commercially available software SPSS (StatisticalPackagefor Social Sciences) Version 20.1 (Chicago, USA Inc.). A subject level analysis was performed for each of the parameters. Changes were seen in theprimaryclinicaloutcome variables – Clinical Attachment Loss and Pocket Probing Depth. Changeswerealsoseen in Secondaryclinicaloutcome variables - Gingival and Plaque Indices. Mean Standard Deviation fortheclinical variables were calculated for each treatment. The Kolmogorov Smirnov test was used tochecknormailty of data and independant sample t-test and paired t-test wasusedtocheckthe mean differnces between groups where appropriate. The effectsize wa scalculatedusing Cohen's D formula.

The following formula is involved in the calculation of Cohen's d effect size values for t-tests:

Cohen's d effect size for a t-test

$$d = \frac{|x_1 - x_2|}{\sqrt{(\sigma_1^2 + \sigma_2^2)/2}}$$

where x_1 and x_2 are the means of group 1 and group 2, and σ_1^2 and σ_2^2 are the variances of group 1 and group 2.

Interpretation:

≤ 0.20 is a small effect size, 0.50 is a moderate effect size and ≥ 0.80 is a large effect size

RESULTS

Intra group Comparison: Gingival Index: The values for Gingival Index in Group A were 1.51 at baseline, 1.33 at 1 month and 1.21 at 3 months. The difference of baseline to 1 month was statistically significant (p value 0.000). The difference from 1 month to 3 months was also found to be significant (p value 0.003). The values for Gingival Index in Group B were 1.46 at baseline, 1.19 at 1 month and 1.02 at 3 months. The difference between these values was found to be statistically significant (p value 0.000 for both).

Plaque Index: The values for Plaque Index in group A were 2.17, 1.97 and 1.80 at baseline, 1 month and 3 months respectively. The difference was found to be statistically significant at $p<0.01$ (p value 0.000). The values of Plaque Index in Group B were 2.13, 1.92 and 1.78 at baseline, 1 month and 3 months respectively. The difference was found to be statistically significant at $p<0.01$ (p value 0.000).

Pocket Probing Depth: The mean probing depth of Group A was 6.10 at baseline and 4.50 at 3 months which was found to be statistically significant (p value 0.000) with the effect size of 0.666. (Table 6). The mean probing depth for Group B was 6.15 and 3.90 at baseline and 3 months respectively which was found to be statistically significant (p value 0.000) with the effect size of 0.793.

Clinical Attachment Loss: The mean CAL for Group A was 5.15 and 3.55 at Baseline and 3 months respectively. The difference was statistically significant at $p < 0.01$ (p value 0.000) with an effect size of 0.730. The mean CAL for Group B was 5.10 and 2.80 at Baseline and 3 months respectively and the difference was statistically significant at $p < 0.01$ (p value 0.000) with an effect size of 0.828.

Intergroup Comparison

Gingival Index: The Gingival Index scores were found to be 1.51 and 1.46 at Baseline for Group A and Group B respectively and the difference was not statistically significant. At 1 month, the mean scores were 1.33 and 1.19 and at 3 months they were 1.21 and 1.02. although the mean scores seemed to reduce for the both groups over the study period, the difference was not statistically significant (p value 0.708, 0.291 and 0.114 at baseline, 1 month and 3 months respectively).

Plaque Index: The Plaque Index scores were found to be 2.17 and 2.13 at baseline, 1.97 and 1.92 at 1 month and 1.80 and 1.78 at 3 months for Group A and Group B respectively. The reduction of the mean scores, when compared was not statistically significant (p value 0.804, 0.708 and 0.854 at baseline, 1 month and 3 months respectively).

Probing Pocket Depth: The mean periodontal pocket probing depth was 6.10 and 6.15 for Group A and Group B respectively, at baseline, with no statistically significant difference (p value 0.856). At 3 months, the mean probing depth reduced to 4.50 for Group A and 3.90 for Group B.

Table 1. Mean values of gingival index, plaque index, periodontal pocket depth and clinical attachment loss between the two groups – Chorion and Chorion with Metronidazole

Variables	Chorion (n=20)	Chorionwith 2% Metronidazole (n=20)
Gingival Index		
Baseline	1.51 ±0.49	1.46 ±0.32
1 Month	1.33 ±0.45	1.19 ±0.33
3 Months	1.21 ±0.41	1.02 ±0.34
Plaque Index		
Baseline	2.17±0.54	2.13 ±0.46
1 Month	1.97 ±0.51	1.92±0.40
3 Months	1.80±0.46	1.78 ±0.38
Periodontal Pocket Depth		
Baseline	6.10 ±0.91	6.15±0.81
3 Months	4.50 ±0.88	3.90 ±0.91
ClinicalAttachmentLoss		
Baseline	5.15 ±0.67	5.10 ±0.55
3 Months	3.55±0.82	2.80 ±0.95

This difference was statistically significant at $p < 0.05$ (p value 0.042)

Clinical Attachment Loss: The mean values of CAL were 5.15 and 5.10 at Baseline for Group A and Group B respectively. And the difference was not statistically significant. At 3 months, the mean values reduced to 3.55 for Group A and 2.80 for Group B and the difference was found to be statistically significant at $p< 0.05$ (p value 0.011)

DISCUSSION

The evaluation of the efficacy of Chorion membrane with and without 2% Metronidazole gel for periodontal pocket therapy was done based on clinical parameters only. The clinical parameters used were a set of two indices – the Plaque Index and the Gingival Index and a set of two measurements – Pocket Probing Depth and Clinical Attachment Loss. The plaque index used was the Turesky-Gilmore-Glickman Modification of Quigley-Hein Plaque Index (1970)[23]. This index was used as it has more objective definition of scoring because of the use of a disclosing agent to identify the plaque and a more objective definition of each numerical score. Also, the repeated evaluation of the plaque scores can be seen as a tool to monitor patient compliance. The Gingival Index used was the modification of Loe and Silness Gingival Index (1967)[23]. The index shows good validity, reliability and ease of use and also demonstrates sufficient sensitivity to distinguish between groups of mild and severe gingivitis. It also gives quantitative measurements of the bleeding scores. For research and clinical trials, a quantitative measurement of bleeding is more important than a dichotomous index of presence or absence of bleeding on stimulation. Pocket probing is one of the important clinical features of periodontitis, therefore, measure of pocket depth becomes important in evaluation of the progression or regression of periodontal disease post treatment. However, since pocket depth is dependent on the position of the gingival margin, its position may change from time to time which changes in the level of the gingival margin without giving the actual gain in the attachment. Thus the level of attachment which is the distance from a fixed point on the crown to the base of the pocket is a better indicator of periodontal destruction.

In the present study, the cement-enamel junction was considered to be the fixed point. Clinical Attachment Loss is viewed as the gold standard when looking at the effects of treatments designed to improve chronic periodontitis. The fixed point generally taken in the measurement of CAL is a fixed point on the crown of the tooth, like the cement-enamel junction or the margin of restorations. Phase I therapy was carried out in all the patients to eliminate all the possible etiological factors responsible for periodontal disease progression and pocket formation. It also helped in bringing back the inflamed gingival tissues to the normal consistency which did help in surgical manageability of the gingival tissue. Although there is a scarcity of literary evidence on the efficacy of Chorion membrane in the elimination of periodontal pockets, it has been proved to be beneficial in treating gingival recession defects[24]. However, the results of this study are in accordance with a similar experiment conducted by Kothiwale S (2014)[25] who also found an improvement in the clinical parameters. The main advantage of this technique is that it does not require a second surgical procedure thus improving the long term clinical results without disrupting the attachment. The significant reduction in the pocket probing depth may be credited for the presence of tissue inhibitor of matrix metalloproteinases (TIMPs) in chorion membrane which suppresses matrix metalloproteinases and transforming growth factor beta (TGF-β) which stimulates the production of TIMPs from the surrounding tissue (Riau *et al* 2010)[26]. Collectively these proteins suppress inflammation and degradation of collagen. Also, the presence of intense concentrations of laminin and laminin-5 throughout the barrier is of particular importance due to its high affinity for binding gingival epithelial cells which may contribute for better adaptation to the root surface. (Pakkala *et al* 2002). Also, the better results obtained in Group B (Chorion Membrane with 2% Metronidazole gel) were in accordance with studies conducted Mohindra *et al* (2014) who stated that the improvement of clinical parameters was due to the fact that Metronidazole reduced or inhibited the penetration of micro-organisms through the guided tissue regeneration membrane. Moreover, the selectively bactericidal nature of Metronidazole towards obligate anaerobes could also be a contributing factor. Carranza defined repair as 'healing by scar' of which disease progression is halted. There is no increase in bone height. The

destroyed periodontium is replaced by mobilization of epithelial and connective tissue cells into damaged area and increased local mitotic divisions to provide the sufficient number of cells (Newman, 2006). Although, the treatment of vertical bone defects has been studied in detail, there is no long term evidence about treatment of horizontal bone loss. Various methods have been experimented for improvement of alveolar bone level which includes use of systemic antibiotics, anti-inflammatory drugs, bisphosphonates, rhBMP-2, use of DFDBA in combination with Guided Tissue Regeneration, use of large membranes to cover extensive periodontal defects, adjunctive use of Enamel Matrix Protein, Guided Tissue Regeneration and coronally anchored flaps, supracrestal placement of Tricalcium phosphate ceramic – microfibrillar collagen, composite graft in animals and space provision by reinforced ePTFE membrane. If the horizontal bone loss is not very severe, pocket elimination surgery has been attempted by osteoplasty and creating most readily maintainable contours.All these procedures attempted have given mixed and sometimes discouraging results concluding that horizontal bone loss cannot be treated in a predictable way today (Jayakumar, 2010). In supracrestal periodontal defect, new attachment formation is entirely dependent on the coronal growth of the periodontal ligament cells from the apical portion of the wound. Conversely, in angular defects, the lateral borders of the defect may also provide a source for granulation tissue formation. Therefore, horizontal bone loss is characterized by a very low predictability of the results when treated by regenerative procedures (Jayakumar, 2010). Blumenthal *et al* (1988)[31] stated that collagen membrane supports a dense connective tissue matrix and also act as a scaffold for connective tissue invading the wound. The soft tissue root interface probably consists of a long junctional epithelial adhesion (Blumenthal, 1989). It is generally accepted that after suprabony defects are treated, the gain in clinical healing is the result of epithelial and connective tissue adhesion to the root surface (Nemcovsky, 2006). It is possible to hypothesize that the greater CAL gain observed in this study may be the consequence of supracrestal connective periodontal attachment (Nemcovsky *et al*., 2006 and Stahl, 1991).

Conclusion

The findings of this study suggested that placement of Chorion membrane with 2% Metronidazole gel resulted in significant improvement in the clinical parameters when compared to placement of Chorion membrane alone. However, further long-term studies can evaluate the regenerative capabilities of this material by histological methods or surgical re-entry. These studies should determine the efficacy of Chorion membrane as well as investigate to what extent it may exert an influence on the healing dynamics following periodontal procedures.

REFERENCES

Blumenthal N. 1988. The effect of supracrestal tricalcium phosphate ceramic microfibrillar collagen grafting on postsurgical soft tissue level. *J Periodontol.,*59(1):18-22.

Buayaratavej P, Wang HL. 2001. Collagen memnrabes: A review. *J Periodontol.,* 72(2):215-229.

Darveau RP, Tanner A, Page RC. 2000. The microbial challenge in periodontitis. *Periodontol.,* 1997; 14: 12-32.

De Sanctis M, Zucchelli G, Clauser C. 1996. Bacterial colonization of bioabsorbable barrier material and periodontal regeneration. *J Periodontal Nov.,* 67(11):1193-1200.

Dr. Bui DX. New attachment and reattachment in periodontal therapy. www.drbui.com/artattachment.html.

Esteves J, Bhat KM, Thomas B, Varghese JM, Jadhav T. 2015. efficacy of human chorion membrane allograft for recession coverage: A case series. *J periodontal.,* 86(8):941-944.

Flemming T. 1999. Periodontitis. *Ann Periodontol*; 4: 1-6.

Ghayoumi N. 2001. The use of metronidazole in the treatment of periodontal diseases. *J west Soc Periodontol Abstr.,* 49(2):37-40.

Haffajee AD, Socransky SS. 2000. Microbial etiological agents of destructive periodontal diseases. *Periodontol*, 1994; 5: 78-111.

Haffajee AD, Socransky SS. 2005. Periodontal microbial ecology. *Periodontol* 2000, 38: 135-187.

Jayakumar A, Rohini S, Naveen A, Haritha A, Reddy K. 2010. Horizontal bone loss: A periodontal orphan. *J Indian Soc Periodontol.,*14(3):181-183.

Kornman KS, Robertson PB. 2000. Fundamental principles affecting the outcomes of therapy for osseous lesions. *Periodontol* 22:22-43.

Kothiwale SV. 2014. The evaluation of chorionic membrane in guided tissue regeneration for periodontal pocket therapy: A clinical and radiographic study. *Cell Tissue Bank.,* 15:145-152.

Loe H, Theilde E, Jensen SB. 1965. Experimental gingivitis in man. *J Periodontol.,* 36: 177-187.

Mohindra K, Malhotra R, Grover V, Grover D. 2014. Bone regeneration using metronidazole containing collagen sponge in periodontal horizontal bone defect. *Indian J Oral Sci.,*5:128-133.

Mombelli A, Lang NP, Nyman S. 1993. Isolation of periodontal species after guided tissue regeneration. *J Periodontol* Nov., 64(115):1171-1175.

Moore WE, Moore LV. 2000. The bacteria of periodontal diseases. *Periodontol* 1994; 5: 66-77.

Nemcovsky CE, Zahavi S, Moses O, Kebudi E, Artzi Z, Beny L, Weinrev M. 2006. Effect of enamel matrix protein derivative on healing of surgical supra-infrabony periodontal defects in the rat molar. *J Periodontol.,* 77(6):996-1002

Newman MG, Takei HH, Klokkevold PR, Carranza FA. 2006. Carranza's clinical periodontology, 10th Edition. *Elsevier* Pte.Ltd.

Page RC, Offenbacher S, Schroeder B, Seymour G, Kornman K. 1997. Advances in the pathogenesis in periodontitis: Summary of developments, clinical implications and future directions. *Periodontal* 2000, 14: 216- 218.

Pakkala T, Virtanen I, Okasanen J, Jones JCR, Hormia M. 2002. Function of laminins and laminin binding integrins in gingival epithelial cell adhesion. *J Periodontol.,* 40:709-719.

Raja S, Byakod G, Pudakalkatti P. 2009. Growth factors in periodontal regeneration. *Int J Dent Hyg.,* 7(2):82-89.

Riau AK, Beverman RW, Lim LS, Mehta JS. 2010. Preservation, sterilization and de-epithelialization of human amniotic membrane for use in ocular surface reconstruction. *Biomaterials.,* 31:216-225.

Sam G, Pillai BRM. 2014. Evolution of barrier membranes in periodontal regeneration. Are the third generation membranes really here? *J Clin Diagn Res.,* 8(12):ZE14-ZE17.

Sela MN, Babitski E, Steinberg D, Kohavi D, Rosen G. 2009. Degradation of collagen guided tissue regeneration membranes by proteolytic enzymes of Porphyromonasgingivalis and its inhibition by antibacterial agents. *Clin Oral Implants Res.,* 20:496-502.

Sgalastra F, Gatto R, Petricci A, Monaco A. 2012. Effectiveness of systemic amoxicillin/metronidazole as adjunctive therapy to scaling and root planing in the treatment of chronic periodontitis: A systematic review and meta-analysis. *J Periodontol.,* 83(10):1257-1269.

Slots J. 2002. Selection of antimicrobial agents in periodontal therapy. *J Periodontol Res*.,37(5):389-398.

Soben Peter. 2007. Essentials of preventive and community dentistry, 3rd Edition. *Arya Publishing House*.

Stahl SS, Froum SJ. 1991. Healing of human suprabony lesions treated with guided tissue regeneration and coronally anchored flaps. *J Clin Periodontol.,*18:69-74.

Tomas I, Tomas M, Alvarez M *et al*. 2007. Susceptibility of oral obligate anaerobes to telithromycin, moxifloxacin and a number of commonly used antobacterials, *Oral Microbiol Immunol.,*22:298-303.

Yoshinari N, Tohya T, Mori A, Koide M, Kawase H, Takada T, Inagaki K, Noguchi T.1998. Inflammatory cell population and bacterial contamination of membranes used for guided tissue regeneration procedures. *J Periodontol Apr*; 69(4)460-469.

Yu L, Liu H, Lingling E, Wu X, Wang D. 2009. Uptake of metronidazole by human fibroblasts. *J Periodontol*; 80(6):993-998.

Zandbergen D, Slot DE *et al*. 2004. The clinical effect of scaling and root planing and the concomitant administration of systemic amoxicillin and metronidazole: A systematic review. *J Periodontol.,* 84(3):332-351.

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