Research Article

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Metronidazole Gel foam Influence on Salivary Tumor Necrosis Factor and Interleakin-6 with Lower Third Molar Extraction (Randomized Control Clinical Study)

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ABSTRACT

***Aim**: Estimation of influence of metronidazole gel foam on Salivary Tumor Necrosis Factor and Interleukin-6 during lower third molar extraction by comparing the effect before and after extraction. In addition, evaluate the benefit of using metronidazole gel foam during the extraction of molar teeth.*

***Material and Methods**: Twenty three patients were involved in this study, patients were classified into group I (control group) group II (metronidazole gel foam tested group), saliva was taken from all patients by using salivate before extraction and seven days after extraction to measure the TNF-α and IL-6 by using (ELISA) kit.*

***Results**: there is a significant decrease in the salivary interleukin-6 and TNF-α levels before extraction and after one week in both groups ($p<0.05$), negative correlation exist between salivary TNF-α and IL-6 in both groups, also there is no significant difference of the level of TNF-α between two groups ($p=0.078$), and a significant difference in the level of IL-6 ($p=0.018$) between two groups.*

***Conclusion**: metronidazole gel foam produces a significant effect on salivary TNF-α and IL-6 when compared to its level in control and treatment group which may enhance healing of socket after extraction of lower third molar and decrease susceptibility of infection.*

Keywords

Metronidazole Gelfoam, Lower Third Molar Extraction, Salivary Inflammatory Biomarker, Saliva ELISA Test.

Introduction

Extraction is a main simple procedure that is performed popularly in the dental clinic, the consequences of this "simple" procedure have not always been accurately assessed, each individual has his own capacity to heal, which is determined by his biotype and biological profile, consisting of cytokines and inflammation mediators [1]. Healing takes place in three stages, an inflammatory phase, a proliferative phase, and a maturation phase, the first process initiated as soon as blood platelets come into contact with collagen, connective tissue while blood fills in the empty socket, this creates a platelet aggregation that forms a clot (erythrocytes and leukocytes embedded in fibrin gel), the clot controls the bleeding and considers as a support to the successive stages of cicatrization, scientifically platelets produce growth factor and mediators (cytokines) involved in angiogenesis [2]; cytokines are soluble proteins that play an important role in the initiation and

maintenance of inflammatory and immune response as well as intracellular cross-talking [3].

Interleukin-6 is a multifunctional cytokine synthesized in response to stimuli such as infection and traumatic injuries by a variety of cells such as macrophages, neutrophils keratinocytes, fibroblast and endothelial cell [4]. Saliva is a completely unique oral fluid produced form primary and minor salivary gland, it may be used to offer clinical statistics about patients rapid increase, in the used of saliva as a diagnostic medium within the last few years, it can be performed by measuring antibodies and protein concentration, flowed of saliva [5].

Gelfoam sterile sponge is a medical device intended for application to bleeding surfaces as hemostatic, metronidazole has a well-documented effect on the prevention and treatment of anaerobic infection [6]. Gelfoam with sterile metronidazole solution was effective in the reduction of the inflammatory process after extraction of lower third molar this lead to a question, which is the effect of metronidazole gel foam on the healing process, after extraction, or as the result of its effects on inflammatory biomarkers like tumor necrosis factor and interleukin-6 before and after treatment. From this point of view, authors cooperate to estimate the influence of metronidazole gel foam on salivary Tumor Necrosis Factor (TNF) and Interleukin-6 (IL-6) during lower third molar extraction by comparing the effect before and after extraction. In addition, evaluate the benefit of using metronidazole gel foam during the extraction of molar teeth.

Materials and Methods

Ethical Approval

All work prepared According to the CONSORT 2010 checklist. It's approved by the scientific committee of Nineveh Health Directory / MOH / Iraq.

This study was carried out at Al-Noor Specialist Dental Center/ Department of Mosul Iraq in the period from February 2020 to August 2020. Total patients included in this study were (36), only (23) patients we eligible to be included in this study, (18) female, (5) male. Inclusion criteria were healthy patient need simple extraction of lower third molar teeth, no concomitant disease, non-smoker or alcoholic. As well as lacking allergic history to any of our tested materials, no previous history of any complication. No history of any medication uses previous to extraction. Finally, patients weren't currently on a prescribed drug for the last six months.

Randomization and Grouping Patients

Group I (Control Group CG): twelve patients underwent extraction of the lower third molar smoothly without gel foam placement in the socket after extraction. Patients received simple instructions and prescribed analgesics as Paracetamol tablets (500 mg) on need. In addition, Antibiotic drug as Augmentin tablets (625 mg). Advised to be seen within seven days to asked them to return to their dentist if any signs of dry socket are appeared as (bleeding or severe pain).

Group II (Tested Group TG): includes eleven patients were gel foam with metronidazole placed in the socket after extraction of the lower third molar. Lincomycin gel foam was placed in the socket and the patients received simple instructions and use of simple analgesics as paracetamol tablets (500 mg) on need. In addition, Antibiotic drug as Augmentin tablets (625 mg), and asked them to return to their dentist within seven days if any signs of dry socket are appeared as (bleeding or severe pain).

Saliva Examination

Salivary secretion is gathered from all patients in both groups before extraction and at the seventh day after extraction by using salivate, then saliva centrifuged at 3000 rpm for 10 minutes according to T. AL-Sandook instruction in their published article [7], and freeze in enpindrofe, finally analyzed by Using (ELISA) kit (SALMETIX SALIVARV IL-6, MY BIOSOURCE.TNF-α) to determine the level of (TNF-α) and (IL-6).

Statistical analysis

Microsoft Excel -2010 was used for data categorization and coding. Descriptive and analytic statics was performed using the Minitab version 18 software statistic program. The data were expressed as mean ± SD difference between two experimental groups were statically analyzed the level of significance was at $P<0.05$ by using paired T-test of two mean.

Results

Patients aged from 18 to 45 years with a mean (40.5 years). The effect of metronidazole gel foam on mean salivary levels of TNF-α and IL-6 in Group II after one week was shown in Table 1, which revealed the comparison between salivary parameters before extraction and after extraction by using lincomycin gel foam the results were showed there are no significance differences of TNF-α with P-value 0.060 and the mean ± SD is 15.6 ± 0.04 while the IL-6 there is significance differences with P-value is 0.014 and mean ± SD is 5.36 ± 4.93

Table 1: Effect of metronidazole gel foam on mean salivary levels of TNF-α and IL-6 after one week.

Salivary parameters	Group II (TG metronidazole gel foam)		P-value
	Beginning mean ±SD	After one week mean ±SD	
TNF-α (pg/ml)	106.0±141.5	15.6±0.04	0.010
IL-6 (pg/ml)	1.74±0.92	5.36±4.93	0.014

Table 2, that revealed the mean and standard deviation for salivary parameters IL-6 and TNF at the beginning of the study and after one week in the control group which showed that there is a significant difference in salivary IL-6 after one week (P-0.002) mean ±SD is (56.8 ± 79.3) and significance difference with salivary TNF-α with (P= 0.024) and mean ±SD is (56.8 ± 79.3)

Table 2: Changes in mean salivary levels of TNF-α and IL-6 of the control group.

Salivary parameters	Control group (JP)		P-value
	Beginning mean ±SD	After one week	
TNF-α	102.9 ± 133.3	56.8 ± 79.3	0.024
IL-6	1.57 ± 0.18	16.99 ± 13.31	0.002

Table 3, this table revealed the correlation matrix between TNF-α and IL-6 in the metronidazole gel foam group at the end of the study by using Pearson Correlation that revealed a negative correlation between IL-6 and TNF-α. As same as a negative correlation between IL-6 and TNF-α in the control group at the end of the study by using person correlation also confirmed as presented in Table 4.

Table 3: Correlation between TNF-α and IL-6 in group II.

Parameters	Correlation coefficient	TNF-α
IL-6	R	-0.359
	P	0.278

Table 4: Correlation between TNF-α and IL-6 in group I.

Parameters	Correlation coefficient	TNF-α
IL-6	R	-0.462
	P	0.131

Table 5, this table revealed the comparison in salivary levels of TNF-α and IL-6 among the two groups at the end of the study by using the one-way ANOVA-test. Which demonstrate that there is no significant difference in the level of TNF-α between two groups (p=0.078), on the contrary significant difference in the level of IL-6 (p=0.018) between two groups.

Table 5: Level of TNF-α and IL-6in group I and II after one week.

Parameters	Group I (CG) mean ± SD	Group II (TG) mean ± SD	P-value
TNF-α	56.8±79.3	15.6±0.04	0.078
IL-6	16.99±13.31	5.36±4.93	0.018

Discussion

The consequences of the extraction procedure have not always been accurately assessed, whereas many mucosal and osseous complications may show up after the extraction: bone resorption with the collapse of the alveolar process, a gingival cleft or gingival recession in the area surrounding the extraction site [8].

According to the result of this study, the level of TNF-α in the control group and lincomycin tested group showed decreases in value from the beginning of the study and seventh day after extraction with a significant difference (group 1) and no significant difference (group2).

TNF-a is expressed on activated macrophages and lymphocytes as well as other cell types, it is a potent pro-inflammatory cytokine exerting pleiotropic effects on various cell types and plays a critical role in the pathogenesis of chronic inflammatory diseases, such as RA [9].

Each individual has his own capacity to heal which is determined by his biotype and biological profile consisting of cytokines and inflammation mediators. TNF is capable of acting independently or in conjunction with a wide range of other factors. It exists in 2 forms: TNF α and TNF β. TNF α is produced by activated macrophages, though much less by other cell types, TNF α and TNF β bind to the same receptors. They both have a half-life of 15-18 minutes, yet indicating metabolic and hemodynamic changes. There is a presence of endogenous inhibitors (trans-membrane soluble TNF receptors-STNFRs) which inhibit the potential unregulated TNF activity.

TNF α has cytotoxic effects on endothelial cells, it increases the adhesion of neutrophils to endothelial cells through regulation of adhesive molecules [7] and increases vascular permeability, both directly and indirectly, through activation of neutrophils. It helps the release of prostaglandin PGE2, it activates PAF (thrombocyte activating factor) and the coagulation process. TNF and IL-1 are the most important inducers of acute phase response (3) that is why it's high at the beginning of our study than seventh days after the extraction.

According to the result of this study, the level of IL-6 in the control group (group one) and lincomycin group (group two) showed an increase in value from the beginning of the study and seventh day after extraction with a significant difference in both groups.

IL-6 has a molecular mass of 26 kD, its main features include synergism with IL-1 and TNF for the purpose of co-stimulating immunological response and inducing the production of acute-phase proteins. It has a short life span of about 1 hour following the occurrence of a trauma, its values can be detected after 1 hour, the concentration peak is reached after 4-6 hours and it can be present for 10 days in circulation. This cytokine is constantly detected in plasma, which suggests its constant production. It is also created in phagocytes, the vascular endothelial cells and the fibroblasts. IL-6 enables B-cell replication, differentiation and immunoglobulin production. IL-6 has the role of mediators of the acute phase response (CRP, fibrinogen, haptoglobin, amyloid α, α-1- antitrypsin and complement activation-C3 and factor B). It possesses imunomodulatory characteristics, including initiating PMN (polymorphonuclear leukocytes) - mediated hyper-inflammation and paradoxically, deferred immunosuppression of the host. IL-6 can also serve as an anti-inflammatory mediator through a sophisticated mechanism of releasing STNFS (soluble TNF receptors).

The increased plasma concentrations have been registered in acute states, such as surgical action of TNF-αl interventions, burns and bacterial infections. IL- 6 is closely associated with the events occurring in the post-operative period (elective surgery) or following trauma, with the increased levels of IL-6 48 hours postoperatively constituting an alarm of possible infective postoperative complications, present immunosuppression or representing a reaction of the organism to a hyper-inflammatory condition.

There is a significant correlation between IL-6 values and age. Still, IL-6 remains the cytokine that is most constantly elevated or most easily detectible [10]. According to this study, there is a negative correlation between TNF and IL-6which is agrees with the study of Matsuno et al. [11], who showed that TNF-α can increase the production of IL-6 while IL-6 in contrast, does not increase the production of TNF-α in vivo and it's also proved in the previous study *in vitro* [11].

According to this study, metronidazole provides a highly significant effect on TNF-α and IL-6 when compared with control group, metronidazole belongs to the effective anaerobic group of antibiotics and is widely used to treat bacterial infections caused by and most Staphylococcus types [12]. Antibiotics penetrate more effectively to the skin and bones, as well as most tissues.

Conclusion

Metronidazole gel foam produces a significant effect on salivary TNF-α and IL-6 when compared its level in control and treatment group which may enhance healing of socket after extraction of lower third molar and decrease susceptibility of infection.

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