

Salivary Interleukin – 11 Levels in Multiple Teeth Implants Patients Before and After Suture Removal (Comparative Study)

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ABSTRACT

Introduction: Interleukin-11 (IL-11) is a cytokine with many functions as it has a significant role in the inflammatory processes of the dental tissue. Also, It has been stated to have a role in inhibiting the pro-inflammatory cytokines' production such as IL-1 β , tumor necrosis factor α , IL-6.

Objective: To investigate the levels of interleukin 11 (IL-11) are before and after removal of sutures of dental implants which are used as fixators for dental prostheses.

Materials and Methods: A random comparative cross-sectional study were done on dental implant patients whose age ranges between 30-40 years old who were free from any systemic diseases and were non-smokers at Isra University Dentistry College, Baghdad, Iraq. Among 40 patients selected as the study sample, 2 samples were taken from each patient, as the study was done in 2 phases, one phase prior to and one phase after removal of sutures, 40 samples were in study (A) in which their samples were taken after implant placement immediately by taking 5 ml of each patient saliva, while the other 40 samples in study (B) of the same patients were taken after removal of the sutures, 10 days after taking the first samples. For our study, 2 to 4 Implants were used for each patient and many flap suturing techniques were used, the system implant used was of 'ot-medical' type which was made in Germany and Interleukin-11 bioMérieux kit used which was made in France, levels of IL-11 were

assessed in the samples by immunosorbent enzyme-linked assay. SPSS version 16 was used for statistical analysis.

Results: The values of interleukin-11 were significantly higher in the samples study (A) after inserting the implants than in the samples of study (B). The concentration of Interleukin-11 were in higher in study (A) because of the aggressive inflammatory process due to insertion of the dental implants.

Discussion: The levels of Interleukin-11 are much more after inserting the implant compared to after removal of the suture due to alleviation of the inflammatory condition (P<0.003).

Conclusion: Interleukin-11 acts an anti-inflammatory cytokine, so after insertion of the implant, being a foreign body, the IL-11 highly increases compared to reduction of the inflammatory process by removal of the sutures.

Keywords: Salivary Interleukin-11, dental implants, Sutures, Comparative Study.

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INTRODUCTION

Interleukin-11 was identified originally depending on its biologic resemblances to interleukin-6, however, those 2 cytokines, are not identical in their functions. Characterization and Identification of the components of the receptor of interleukin-11 and its related signal molecules are needed for dissecting the mechanisms which are responsible for specificity of that cytokine.

Interleukin-11 is a cytokine which have a limited targeted cells' spectrum than interleukin-6 and that specific property might justify the least or the non-toxicity related to interleukin-11 in the studies of in vivo. Administration of interleukin-11 in vivo in bone marrow depressed and normal animals has stated the thrombopoietic impact of that cytokine. (1)

Due to its activity of immunomodulation and its capability of promoting the mucosal healing of the gastrointestinal tract, interleukin-11 may reduce also the aggressiveness of mucositis induced by chemotherapy and its inflammatory related complications. Those biologic activities have resulted in carrying out of many clinical trials for treating Crohn's disease and mucositis induced by chemotherapy.

Besides to, depending on its capability of enhancing thrombopoiesis, interleukin-11 is being more assessed in the cure of many inflammatory diseases as sepsis, inflammatory bowel disorder and rheumatoid arthritis. The upcoming clinical trials with only interleukin-11 and combined with other radiation or chemotherapeutic and

biological agents will be significant for evaluating the interleukin-11 efficacy in many clinical issues. (2)

The 'cDNA' which encodes for interleukin-11 was isolated originally from stromal cell line of fibroblasts of bone marrow of a primate depending on its activity of mitogenicity on cell line of plasmacytoma of a mouse which is dependent on interleukin-6. Also, similar 'cDNA' was independently isolated by its capability of inhibiting adipogenesis. Subsequently, in vivo, in vitro and clinical trials have stated that Interleukin-11 is a cytokine with many functions which are significant for hematopoiesis. cDNAs of human and mouse receptors of interleukin-11 have been extracted by hybridization by using oligonucleotides degenerated for the receptors of hematopoietin and a method of enhancer trap. (3)

In vivo and in vitro studies have stated that the interleukin-11 is a growth factor having various factors as therapeutic abilities in inducing thrombopoiesis, protecting from injuries of gastrointestinal tract and controlling the immune responses. As various other growth hematopoietic factors, its clinical effectiveness must be interpreted cautiously and might be improved more by being combined with other growth hematopoietic factors.

Genetic Structure of Interleukin-11

The genetic sequence of interleukin-11 is located on a fragment of 7 kb. The human genome includes 4 introns and 5 exons. The promoter of interleukin-11 includes organized binding sites of nucleotides for various transcription factors. Besides to, inducible elements by phorbol ester and interferon have been located on the 59th area of the genome. Two signals for polyadenylation have been found on the 39th end of the genome resulting in generation of 2 transcripts which only differ in the untranslated 39 sequences. (4)

The Structure and functions of Interleukin-11 Protein

Interleukin-11 is unassociated with any different cytokine structurally and shows a number of variable features of proteins as lack of residues of cysteine, sites for glycosylation which is O-linked and glycosylation linked to asparagine.

Interleukin-11 includes large amounts of residues of leucine, proline, and other residues which are positively charged, leading to formation of an extremely basic protein having isoelectric point more than 11.

Interleukin-11 induces in vitro differentiation of osteoblasts. It has been recommended that this differentiation, other than its action of pro-osteoclastogenic, is the dominant impact of interleukin-11 as the mice which overexpress interleukin-11 have elevated formation of bones and thickening of cortex but with no alteration regarding genesis of osteoclasts. There are 2 mechanisms at least by which that happens. (5)

Also, osteoblasts are produced from potent progenitors which are capable of differentiation of adipocytes, and interleukin-11 triggers their commitment towards the osteoblast cell line formation and suppression of adipogenesis. Another mechanism is by which the interleukin-11 inhibits sclerostin expression, which is inhibitor of formation of bones, derived from osteocytes.

Null mice of receptors of interleukin-11 had features which were consistent with all the 3 reported impacts of interleukin-11, that are: reduced genesis of osteoclasts, impaired formation of bones, and enhanced adiposity of bone marrow. That states that signaling of interleukin-11 is significant for those processes. (6)

Null mice of receptors of interleukin-11 as null mice of interleukin-6 have impaired formation of periosteal bone, stating that the impact on differentiation of osteoblasts is not limited to the environment of trabecular bones.

Besides to, interleukin-11 may induce formation of bones responding to mechanical loads. Initial evidence stated that interleukin-11 suppressed the sclerostin gene expression which is sensitive mechanically. Levels of mRNA of interleukin-11 are increased inside bones after exposure to mechanical loads.

Also, 'ΔFosB', which is a member of the 'AP-1 family' which induces differentiation of osteoblasts and is upregulated rapidly with applying mechanical loads, stimulates transcription of interleukin-11 within osteoblasts. The same mechanisms may induce expression of interleukin-11 within osteocytes while exposing to mechanical loads. (5)

After those studies in modified mice genetically and cell culture, significance of receptors of interleukin-11 in human biology was established when mutations in genes of the human receptors of interleukin-11 were stated. Individuals who lack signaling of receptors of interleukin-11 had various symptoms involving abnormalities in teeth, premature fusion of the cranium known as craniosynostosis, and malformation of digits.

Human recombinant interleukin-11 is built within E. coli and varies from the molecule which occurs naturally. A multicentered study was carried out, which aimed at identifying the variable risk factors related to patients of dental implants for success of implants of long term. By using retrospective review of 3 centers, information of patients concerning their sociodemographic variables, and their periodontitis history, besides to the short placement parameters of implant involving implant design, manufacturer, length, location and diameter and placement type, were gathered. (7)

The usage of the common dental implants has become a highly effective and predictable modality of treatment for rehabilitation of partial and complete edentulism. Many studies have stated presence of high rates of survival of 89% to 96% of about 10 years in variable patients by using various systems of dental implants. Furthermore, lower rates but satisfactory success which ranges between 52% to 79% were stated in those studies depending on the selected criteria for assessment of success of implants. (8)

Orthodontic procedures induce physical and chemical responses in the dental tissues. At the start of dental movement of any tooth, the stimulus mechanically leads to a reaction of acute inflammation in the dental tissues, that in turn might induce the biological processes which leads to resorption of bone for accommodating tooth movement. The mechanism of resorption of bone might stimulate the release of many mediators of inflammation, as interleukin-11 and interleukin-1.

Interleukin-11 is produced by different stromal cells, involving epithelial cells, fibroblasts and osteoblasts. It has variable biological functions and activities as for instance: in immune responses, in hematopoiesis, in the metabolism of bones and anti-inflammatory actions. (1)

Interleukin-11 has been stated to possess many properties regarding anti-inflammatory and a polarizing impact of T helper cells type 2 and so it's used to treat most of the inflammatory disorders caused by T helper cells type 1 by applying a modulator impact on T naive cells and on the pro-inflammatory cytokines' expression as interleukin-6, nitric oxide and interleukin-12 produced by macrophages. Besides to, Interleukin-6 down regulates the interleukin-11 expression in cultured cells which are like osteoblasts, and also, it has been reported that expression of interleukin-6 is upregulated significantly by interleukin-17 in synovial, fibroblast and skin cultures. (9)

Localized infections at any site of implants might develop because of poor techniques surgically, contamination or necrosis because of failure in reduction of the numbers of bacteria in the dental area intended for surgery.

There are many evidences that, these infections might be because of infective latent foci activation in the bone, that

are hard to localize in radiological imaging. Also, in spite of being rare, osteomyelitis has been stated after implant placement.

Patients who present with post-surgical infections must be carefully followed up and investigated for preventing the systemic spread of infections. Usage of antibiotic doses repeatedly and blindly must be discouraged strongly.

Regarding dental implants, the treatment of malocclusion of class 2 usually needs retraction and intrusion of the frontal segment, that in turn, often demands reinforcement mechanically of anchorage posteriorly. (10)

Trans-palatal bars, as devices for intra-oral anchorage, might reduce the requirement for elastics of class 2 but also may lead to movement of the mandibular 1st molars to the middle and incisors protrusion.

While, the extra-oral anchorage in a headgear form is rejected usually by patients for professional and social causes. Besides to, if the patient wore the headgear for 14 hours/day, partial anchorage loss maxillary molars movement to the middle are observed also. (10)

The usage of dental implants, mini-screws, micro-screws and miniplates has widely expanded, as those devices supply total anchorage, they are very small so they are placed in any alveolar bone area, and they are easily placed and removed.

Furthermore, the efficacy of micro-screw implants has been stated for controlling anchorage during maxillary frontal teeth retraction, for control vertically of posterior mandibular teeth, and for control vertically of the face profile. But, at the biomolecular level, the micro-screw implants' impact has not been assessed yet.

It was stated that, when failure of implant happens, it's accompanied clinically by increased depth of probing, pain reports from patients, and radiographical loss of bone. That complication has been known as periimplantitis.

It has been presented that, interleukin-11 has a significant role in immune response modulation by reducing the production of proinflammatory cytokines and damage of dental tissues. In spite that, levels of interleukin-1 beta in dental diseases have been extensively studied, only scarce studies have focused on interleukin-11 presence and its functions. (11)

The duration, resolution and intensity of inflammation are based on altering the balance between the pro-inflammatory activities and anti-inflammatory activities during the inflammation of the dental tissue and gingivitis. Many studies have stated that, the cytokines acting against inflammation as interleukin-11 are capable of downregulating the production of cytokines responsible for inducing inflammation from the effector cells.

Also, it has been demonstrated that interleukin-11 in capable of inhibiting the generation of interleukin-1 beta, tumor necrosis factor α , interleukin-6, interleukin-12, nitric oxide and p 40 and downregulates the production of cytokines induced by lipopolysaccharides by inhibition of expression of 'kappa light chain enhancer' in vitro. (12)

The aim of our study is mainly concerned on assessing and comparing between the levels of interleukin-11 after insertion of dental implants and after removal of the sutures which mainly depends on the phases of the

inflammatory process and its severity, production of cytokines, and emphasizing in our study on levels of interleukin-11 mainly.

MATERIALS AND METHODS

This comparative cross-sectional study sample included 40 participants in the age group of 30 to 40 years old from the patients who attended the outpatient clinic, Department of dental implants, Isra University Dentistry College, Baghdad, Iraq, from October 2019 to January 2020.

Ethical approval for carrying out the study was obtained from the Ethical Committee of Medical Center. Informed written consent was gained from all the 40 patients participating in our study.

Inclusion criteria were male and female patients of age group between 30-40 years old, free from any systemic diseases and non-smokers, non-pregnant nor lactating females, giving free consent without any financial or social benefits.

Exclusion criteria were patients younger than 30 or older than 40 years old having systemic diseases as type 1 or 2 diabetes mellitus, cardiac diseases, autoimmune disorders, infections with immunodeficiency virus and hepatitis, smokers, lactating or pregnant females and those who oral contraceptives or any steroids or anti-inflammatory drugs. Also, the participants who had received antibiotics in 6 months duration before the study were excluded.

Regarding the selected 40 participants, 2 samples were taken from each patient, and the study was divided to 2 groups, one group (group A) included salivary samples after inserting the dental implants, while the other group (group B) included salivary samples after removal of the sutures 10 days after taking the first samples, for assessing the levels of interleukin-11 in the 2 groups.

Tools used in the study

For our study, 2 to 4 Implants were used for each patient and many flap suturing techniques were used, the system implant used was of 'ot-medical' type which was made in Germany, then the levels of IL-11 were assessed in the samples by using immunosorbent enzyme-linked assay bioMérieux kit which was made in France.

Evaluation of the participants

All the chosen participants did a full dental examination for the assessment of their condition and their need for inserting implants. Then preparation for collecting the samples was done after informing the patients with all the procedure, the study and its aim and giving them the ability to withdraw from the study whenever they want to.

Procedure for collection of samples

Collection of salivary samples:

The sites for collection of samples were freed from any calculus or debris and was then isolated and dried with rolls of cotton. Also, calibrated and volumetric pipettes with 0 to 10 ml range were used and 10 ml of saliva were collected from each patient, divided into two tubes. The samples were then transferred to vials which contain buffer phosphate saline, then the samples were left to freeze at -70

°C till they were assessed immunosorbent enzyme-linked assay for measuring levels of Interleukin-11.

Assessment of levels of interleukin-11

The procedure of immunosorbent enzyme-linked assay was done at the Microbiology Department, in Isra University Dentistry College, Baghdad, Iraq. The levels of Interleukin-11 were assessed by using an immunosorbent enzyme-linked assay Interleukin-11 bioMérieux kit which is available commercially.

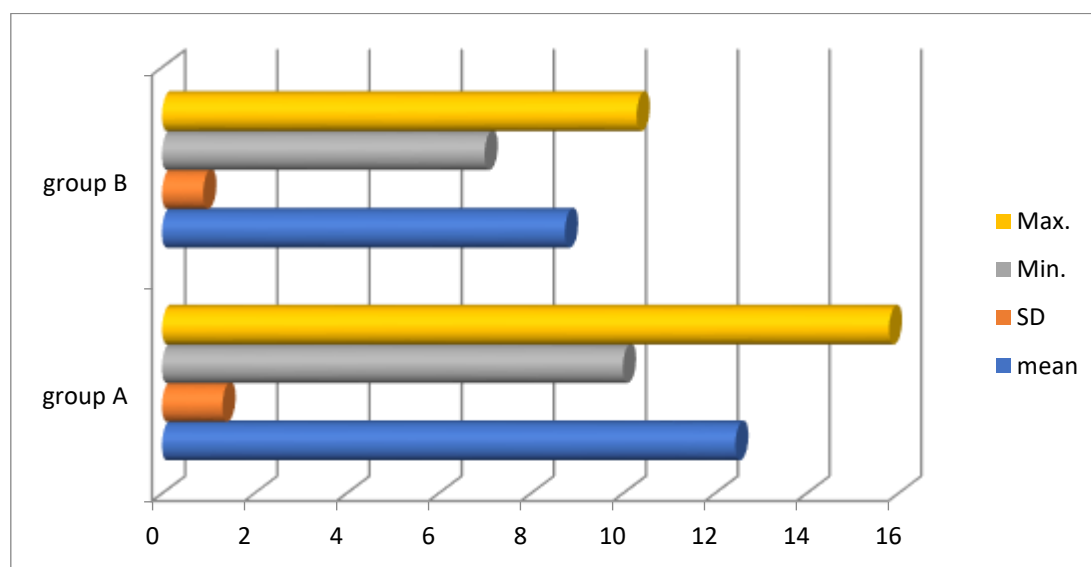
Statistical analysis

All data were analyzed using the SPSS software program version 16, many comparisons for levels of Interleukin-11 using student T test was done for finding out which group is significantly different. Coefficient of Pearson's test was used to investigate any correlation between the levels of interleukin-11 recorded and the presence or absence of the sutures.

RESULTS

Table 1: Descriptive statistics and groups' difference for all parameters

Group	Mean	SD	Min.	Max.	Comparison
Group (A)	12.447	1.310	10	15.8	T-test:-14.328 Pvalue:0.003
Group (B)	8.743	0.894	7	10.3	



Inflammation is normal response of the body to injury of the tissues. The inflammatory responses which cause most of the reactions related to implant placement, range from expected common processes of wound healing to different exaggerated responses of foreign body types and other systemic and local responses.

Being part of the normal immune response of the body, the implant might trigger an acute inflammation of low-level response that is of short period generally, and lasts from minutes up to days, based on the degree of tissue injury related to placement of the implant. (13)

Myeloid cells as macrophages and neutrophils are the initial cells which are involved in the acute inflammation expected with consequent peri-implant healing of wound. No observed complications during the experimental duration involving any fractures of needles, losses of sutures or dehiscences of wound. Manipulation of coated 'polyglactin' and silk were observed to be better than 'poliglecaprone'.

The correlation between the concentrations and the overall amount of interleukin-11 and interleukin-17 and the medical variables at the sites of sampling were studied.

Only significant correlation was found between the overall amount of interleukin-11 and the G-index at the sites of sampling (P= 0.003) and between the overall amount of interleukin-11 and the P-index at the sites of sampling (P=0.001).

DISCUSSION

Department of dental implants is a discipline guided restoratively with a significant surgical section. As placement of implants is usually an optional procedure, it must be planned meticulously and executed for ensuring the highest success probability both aesthetically and functionally with least patient discomfort and morbidity and the least damage risk to the vital organs.

Most significantly, implant placement must not restrict or endanger the future needs for dental cure of the patients. Successful placement of implant must follow the basic oral

surgery principles. It's suitable to divide that to events and phases.

The patient must be informed about what's expected both after and during the surgery and the discomfort level at which they might experience. The patient must consent freely to the implant placement after being told the total information both written and verbally and are supplied with obvious post-surgical instructions involving appropriate management of pain. (14)

Usage of antibiotics after the surgery might not always be mandatory in some cases of good-health patients, however, it's considered significant in grafting of bone or in complex and advanced cases.

The patient is given all the post-surgical comprehensive instructions, appropriate medications for controlling pain and infection prevention, and have a directly twenty-four-hour helpline emergency to the surgeon.

Also, it has been known that, the impact of interleukin-11 in local inflammatory reaction may be related to its impact of suppressing cells so don't release pro-inflammatory cytokines against the action of neutrophils.

Furthermore, a significant role of interleukin-11 and interleukin-17 in preventing destruction of bones which is induced by the pathogens through the neutrophils mobilization has been identified. (15)

In contract to interleukin-11, interleukin-17 is capable of enhancing the proteolytic enzymes activity, as myeloperoxidases, neutrophil proteases, and chemokines and cytokines expression, that facilitates the inflammation development.

Studies which investigate the link between interleukin-11 and inflammatory reactions after placement of implants must be done in large populations for clarifying the association between inflammations and levels of interleukin-11.

CONCLUSION

Healing of wounds is a multifaceted biologic process which includes hemostasis, inflammatory reaction, propagation and tissue remodeling. Huge number of cells involving macrophages, neutrophils, keratinocytes, lymphocytes, endothelial cells and fibroblasts are included in that process.

Many factors may lead to impaired healing of wounds by impacting 1 or more of process phases and are classified into systemic and local factors. The impacts of those factors are not exclusive mutually.

Interleukin-11 is a non-glycosylated peptide which includes 178 amino acids and act as a growth hematopoietic factor and a modulator of the immune responses. Interleukin-11 is a T helper cell type 2-cytokine, which belongs to the interleukin-6 family of cytokines.

It inhibits the synthesis of interleukin-2, interleukin-1, and tumor necrosis factor α and stimulates the release of interleukin-4 and inhibits 'nuclear factor kappa B', that acts as an activator for transcription of pro-inflammatory cytokines.

Interleukin-11 might has a significant role in the immune responses modulation through reducing the production of pro-inflammatory cytokines and damage of dental tissues.

It was stated in our study that, interleukin-11 could be easily detected in salivary samples and the interleukin-11 levels is progressively decreased after removal of sutures compared to its levels after placement of implants immediately.

The reduction in the interleukin-11 levels mostly signifies that both the implant and the sutures have a synergistic role in the induction of the inflammatory responses and production of cytokines.

So, the interleukin-11 is an inflammation biomarker in surgeries of dental implants, which is proved by the alteration of levels of interleukin with the insertion of the implant, insertion of sutures, and removal of the sutures.

More future studies are required for validating interleukin-11 as an inflammation biomarker in surgeries of dental implants and to demonstrate its role in the associating link with production of the inflammatory cytokines.

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