Short Communication

Immunological Study for Interleukin-5 and GM-CSF for Complications Post-Wisdom Teeth Extraction

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Abstract

Background: Wisdom teeth extraction is one of the most common operations conducted in dental clinics and the most common duty performed in oral and maxillofacial surgery clinics. Complications from this treatment are common, including dry socket, postoperative discomfort, delayed healing, postoperative infection, hematoma, edema, and trismus. **Objectives:** The purpose of this study is to quantify the proinflammatory interleukin-5 (IL-5) and granulocyte-macrophage colony-stimulating factor before extraction in saliva by ELIZA technique from patients who visit the clinic to extract wisdom tooth to study its impact on problems following extraction. **Materials and Methods:** A total of 100 saliva study samples (50 cases and 50 control), 44 males and 56 females, aged 19–65 years, were referred to the surgical clinic, College of Dentistry, University of Babylon specialized dentistry centers, and private clinics in Hillah city, Iraq, to determine the levels of preoperative IL-5 and GM-CSF by ELIZA technique. **Results:** There were no major differences between females and males in both groups, according to the findings of this study. In comparison to the healthy subjects, however, patients showed higher significant differences in measuring (IL-5) and (GM-CSF) concentrations (366.81 ± 17.8, 12.26 ± 1.3) ($P \leq 0.05$) by using the ROC test IL-5 and GM-CSF showed (cutoff 146.42, sensitivity 90%, specificity 82%), (cutoff 4.04, sensitivity 84%, specificity 72%). **Conclusion:** proinflammatory IL-5 and GM-CSF were shown to be higher in patients with complications following wisdom teeth extraction than it was in control; highly level of IL-5 and GM-CSF may Predict complications following extraction.

Keywords: Complications, extraction, GM-CSF, IL-5, wisdom tooth

Introduction

Third molar extraction is a common surgical operation in oral and maxillofacial surgery. These teeth should be removed for various causes, including acute or chronic pericoronitis, dental crowding, the emergence of a cyst or tumor, periodontal issues, and caries on the nearby teeth.[1] Complications are more prevalent after wisdom tooth removal than after other teeth removal. This is owing to the anatomical structure of their roots, retention, and (or) dystopia of the wisdom teeth itself, which complicates and makes the surgical intervention to remove them in connection to the soft tissue and bone structures of the jaws more traumatic.[2] Swelling, discomfort, trismus, prolonged bleeding, dry socket, infection, and sensory changes of the inferior alveolar nerve or lingual nerve are all possible postoperative consequences.[3] T cells produce interleukin 5 (IL-5) and IL-3, and some of their actions include proliferation stimulation, differentiation, and survival of myeloid hemopoietic cells, as well as regulating hematopoiesis and inflammation.[4] Cytokines such as IL-1-beta, IL-2, IL-5, IL-6, IL-8, TNF-alpha, and GM-CSF promote vasodilation and leukocyte infiltration of the tissue, which results in the recognizable signs of inflammation. Cells that are stimulated in inflammatory or pathologic situations produce GM-CSF.[5,6]

Materials and Methods

A total of 100 saliva study samples (50 case and 50 control), 44 males and 56 females, aged 19–65 years, who underwent to extract wisdom tooth. All samples were

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collected from the surgical clinic, College of Dentistry, University of Babylon, specialized dentistry centers and private clinics in Hillah City, Iraq. The study extended from November 2022 to January 2023.

Saliva collection

All subjects' mouths were rinsed with distilled water (10 mL) for 30–60 s to ensure that any debris was removed and nonstimulated clean saliva was collected. Saliva collecting by spitting method is collected in the oral cavity and then voided into a receptacle.[7] and stored in a cool box with ice bags to preserve its viability until it was transferred to the laboratory for analysis. All samples were taken to the research facility, put it in a test tube, and centrifuged at (5000 rpm for 15 min). The cleared supernatant was isolated by micropipette to eppendorf tubes and storage them at (-20°C) in the microbiology lab to the point that time of test accumulation was before investigation.

Detecting of IL-5 and GM-CSF by ELISA technique

By using the ELIZA technique, a human IL-5 and GM-CSF-specific antibody has been pre-coated on the microtiter plate included in this kit (Abcam-UK). Standard and samples are placed in the wells of the microtiter plate along with the particular antibody. After that, each microplate well receives sequential additions of a biotinylated detection antibody specific for human IL-5, GM-CSF, and an HPV-conjugated Avidin. Free parts are removed through washing. The substrate solution is poured into each well. There will only be blue coloration in the wells that have human IL-5 and GM-CSF, biotinylated detection antibody, and avidin–HRP conjugate. The enzyme-substrate reaction is halted by adding a stop solution, and the color turns yellow. The optical density was spectrophotometrically measured at a wavelength of 450 nm.

Statistical analysis

SPSS (version 26, SPSS Inc., Chicago, Illinois) was used to analyze the data. Statistics for descriptive purposes (mean, standard deviation), *t*-test student test for comparing between case and control, followed by chi-square. The value of $P \leq 0.05$ was considered to be a statistically significant difference.

Ethical consideration

The ethics of the Helsinki Declaration were followed during the research's execution. Before taking the sample, the patient's verbal and analytical consent were obtained. To obtain this permission, a local ethics committee evaluated and approved the study protocol, subject information, and consent form using document number 6275 (containing the number and date on December 24, 2022).

Results

Distribution of complications following wisdom tooth extraction according to gender

The study includes 100 patients; 50 of them have complications postextraction (28 males and 22 females) and 50 control without complications postextraction (16 males and 34 females) [Table 1].

Determination of salivary IL-5 and GM-CSF concentration

The results showed that the patients' IL-5 concentrations were significantly different from those in the control group ($P \leq 0.05$) [Table 2].

Regarding the level of GM-CSF in patients and the control group, the findings revealed that the patients' group differed significantly from the control group ($P \leq 0.05$) [Table 3].

Prediction of the complications by IL-5 and GM-CSF

The result shows the IL-5 and GM-CSF prediction for complications following extraction [Table 4].

Discussion

Table 1 shows that there was no significant difference in disease distribution between males and females. This study contradicted another study,[8] which revealed that gender was found to escalate the risk of postoperative complications and supported by other studies,[9,10] which found that the complication postextraction wisdom teeth were not influenced by gender. The study's findings were separated into two categories: patients and controls. The concentration of IL-5 in patients was higher than the control sample, as seen in Table 2.

There are no direct studies discussed the effect of IL-5 on the complications post wisdom teeth extraction. This study found one of the most complications postextraction was bleeding; a high level of IL-5 may cause delayed wound healing post wisdom teeth extraction. This result is supported by Leitch *et al*.,[11] who found wound healing is slowed down in IL-5 overexpressing mice, and this is accompanied by significantly higher amounts of eosinophils and CD4(+) cells at the wound site, which may worsen the inflammatory response and cause poor wound healing, and contradicted with another study,[12] which found Th2 cells then acquired additional methods to confine or even eject the offending element, producing cytokines such as IL-4, IL-5, IL-10, and IL-13, which improve eosinophil maturation and recruitment, alternative macrophage activation, IgE generation, to mention a few. Through the creation of granulomas and the deposition of a matrix, several of these Th2 actions promote the "walling off" of large bodies, as would be expected given systems designed to seal open wounds. In this study, from the other side, pain is another

Table 1: Samples distribution according to gender

		Count			*P* value
		Status		Total	
		Case	Control		
Sex	Female	22	34	56	0.842
	Male	28	16	44	
Total		50	50	100	

Table 2: Mean and SD in patients and control group

Variable	Study group	No.	Mean ± SD	*P* value
IL_5) pg/mL)	Case	50	386.81 ± 13.8	0.001**
	Control	50	284.05 ± 3.6	

0.001** mean highly significant at 0.001

Table 3: Mean and SD in patients and control groups ($P \leq 0.05$)

Variable	Study group	No.	Mean ± SD	*P* value
GM-CSF)pg/mL)	Case	50	12.26 ± 1.33	0.001**
	Control	50	7.03 ± 0.59	

0.001** mean highly significant at 0.001

Table 4: The best cutoff, sensitivity, and specificity for prediction of the complication following extraction

Parameter	Sensitivity	Specificity	AUC	Cut off	95% confidence	*P*-value
IL-5	0.90	0.82	0.645	146.24	0.537–0.753	0.012
GM-CSF	0.84	0.72	0.655	4.04	0.548–0.762	0.008

Specificity for each marker = true negatives/true negatives + false positives

complication postextraction; this result corresponds with Merriwether *et al.*,[13] who found greater secretion of IL-5 was significantly associated with pain. The concentration of GM-CSF in patients was higher than in the control sample, as seen in Table 3. Pain is one of the problems postextraction. The result of this study agrees with Lee *et al.*[14] and Nicol *et al.*[15], they discovered that (GM-CSF) is well-described in pain caused by inflammation. Delayed healing is another complication following wisdom teeth extraction. The result agrees with Ure *et al.*,[16] who found (GM-CSF) is hypothesized to play a significant role in impaired wound healing and disagree with Rho *et al.*,[17] who found a cytokine known as granulocyte-macrophage colony-stimulating factor (GM-CSF) is crucial for the healing of wounds.

In this study, we used the ROC test to prove that the high levels of IL-5 and GM-CSF are cytokines for complications following wisdom tooth extraction. The result shown in Table 4, the IL-5 cutoff (146.24) for the diagnosis of disease from healthy (sensitivity 90%, specificity 82%, AUC 0.645, CI: 0.537–0.753); we also found IL-5 cutoff value of 146.24 for the differentiation of patient from healthy. At the same time, ROC analysis revealed a GM-CSF cutoff level of 4.04 for differentiation of patients from healthy control groups (sensitivity 84%, specificity 75%, AUC 0.655, CI 0.548–0.762). So it was suggested that the high level of IL-5 and GM-CSF may be helpful in predicting complications post wisdom teeth extraction; this result is supported by Csősz *et al.*,[18] who found IFN-, GM-CSF, and IL-5 are three substances that have the potential to be predictive biomarkers for the appearance of late flap-related trabeculectomy problems, also supported by Dougan *et al.*,[19]who found GM-CSF levels were significantly higher in patients who developed complications after surgery compared to those who did not. Benefits of predictive study for reduced risk of complications and cost saving. The area under the ROC curve gives an idea about the benefit of using the test Figures 1 and 2.[20]

Conclusion

There was no statistically significant difference in the distribution of issues between males and females. The level of IL-5 and GM-CSF was increased with patients who had complications post-wisdom tooth extraction (pain and bleeding) than in control without any problems postextraction, IL-5, and GM-CSF may have the potential as predictive biomarkers for the complicated postsurgical extraction of wisdom teeth.

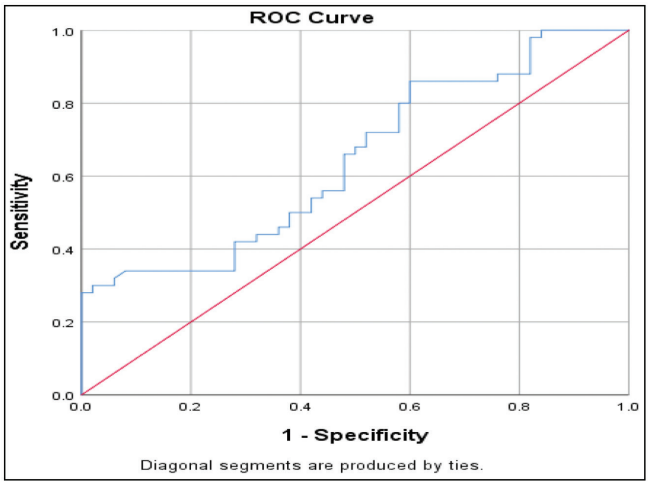

Figure 1: ROC curve for IL-5

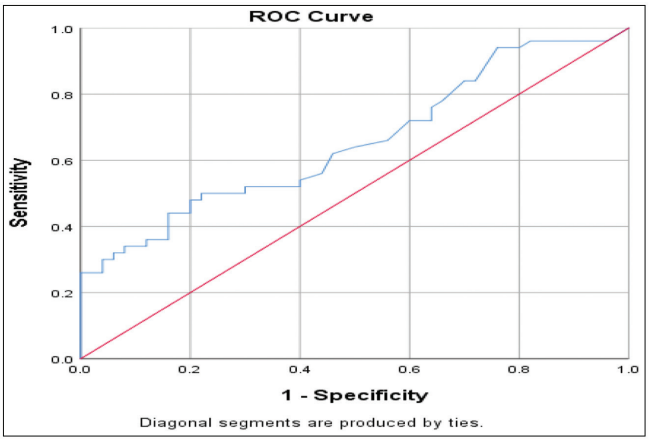

Figure 2: ROC curve for GM-CSF

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Conflicts of interest

There are no conflicts of interest.

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