

Evaluation of Systemic C-reactive Protein as a Systemic Inflammatory Marker in the Blood for Patients Undergoing Minor Oral Surgical Procedures

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ABSTRACT

Background: C-reactive protein (CRP) is an acute phase protein that its plasma levels increase after trauma or surgery so it is used as an indicator for the level of inflammation after surgery. The objective of this study is to investigate pre- and post-operative levels of CRP in three types of oral surgical interventions (Apicoectomy, Impaction, and Impacted teeth exposure).

Materials and Methods: A total number of (48) healthy individuals aged (20-60) years who needed oral surgical intervention for either (removal of impacted third molars, exposure of an impacted canine, or Apicoectomy). A 4ml venous blood was obtained from each patient at two occasions (pre-operatively at the day of operation and post-operatively after 48 hours), then centrifuged for 15 minutes at (1000x g) and finally the sera were separated and stored at (-20° C) to be used for later analysis by Enzyme Linked Immuno Sorbent Assay (ELISA).

Results: There was statistically high significant increase in the level of CRP after oral surgical interventions in all types of operations (the CRP mean value pre-operatively was 2.407925 mg/L, post-operatively after 48hr was 8.486725 mg/L, and the P-value was 0.000). Also there was a high significant difference between apicoectomy, impaction, and exposure operations in the level of CRP at day two postoperatively (P-value was 0.000).

Conclusion: An inflammatory process develops after oral surgical interventions which necessitate the use of anti-inflammatory agents after these procedures and that the severity of inflammation measured by means of CRP levels is correlated to the degree of swelling, length, and type of the surgical procedure.

Key words: CRP, acute phase protein, inflammation after oral surgery. (J Bagh Coll Dentistry 2016; 28(3):110-115).

INTRODUCTION

The common postoperative consequences of the oral surgical interventions are pain and swelling⁽¹⁾. The acute inflammation that evolves as a result of the surgical handling of the soft and hard tissues is the primary cause of these symptoms and signs⁽²⁾. Anti-inflammatory agents are usually prescribed to reduce the postoperative inflammatory sequelae produced by the inflammatory process following these procedures⁽¹⁾. A lot of these anti-inflammatory drugs specially steroidal and non-steroidal anti-inflammatory agents have many side effects and are very toxic to body organs particularly to the liver and kidney. According to American Medical Association Media in each year about 20000 patients die from these drugs and another 100000 patients will end up in the hospital due to liver damage, kidney damage, or intestinal bleeding⁽³⁾. These complications can be randomly predicted by many factors related with oral surgical procedures such as pre-operative inflammation, pre-operative infection, type of operation, time of operation, technique of operation^(4,5).

But these factors alone are not definitive or reliable indicators for the postoperative complications. So the knowledge of reliable indicators of post-operative complications will assess the patient and the operator to manage these complications⁽⁶⁾.

The C-reactive protein(CRP) is an acute phase protein of inflammatory status that increases after surgery or any traumatic situation and in a healthy individual the CRP value is less than 10 mg/L^(7,8).The CRP is produced by the liver in reaction to a variety of stimuli that include damaging of tissues. Small amounts of CRP is found in the blood of healthy persons^(9,10).It has been found that the severity of postoperative outcomes are associated with the level of CRP which is a sensitive biomarker of systemic inflammatory status⁽¹¹⁾. Estimation of inflammatory indicators has been regarded as a way to improve the anticipation of the risk of these outcomes.The aim of this study is to evaluate the CRP levels pre- and post-operatively as a biomarker for the level of inflammation following minor oral surgical interventions so that to make a prediction for the need of use of anti-inflammatory agents with these interventions.

MATERIALS AND METODS

This study was conducted from November 2014 to March 2015 at the department of oral

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and maxillofacial surgery, College of Dentistry, University of Baghdad. It based on clinical, laboratory and radiographical data.

The sample included patients required oral surgical intervention (surgical removal of impacted mandibular third molars, surgical exposure of impacted maxillary canine, or Apicoectomy for maxillary anterior tooth).

Inclusion Criteria

- 1- Adult aged between (20-60) years old of either sex.
- 2- Subjects with good oral hygiene, without stomatitis or any other oral infection.
- 3- Subjects without any systemic infection or acute illness
- 4- Steroids, anti-inflammatory agents should not be taken by the patients in the last week before surgery.

Exclusion Criteria

- 1- Patients with an active infection (local or systemic).
- 2- Medically and immunologically compromised patients such as diabetic patients, endocrine disturbance, anemia, leukemia, other malignant tumors or metastatic diseases.
- 3- Patients with social habits such as alcohol consumption or smoking.
- 4- Patients on steroid therapy or oral contraceptives.
- 5- Pregnant and breast feeding women.

Serum Sample Collection

A 4ml venous blood was collected from each patient at two occasions (pre-operatively at the day of operation and post-operatively after 48 hours), then centrifuged for 15 minutes at (1000x g) and finally the sera were separated and stored at (-20° C) to be used for later analysis by Enzyme Linked Immuno Sorbent Assay (ELISA) for the quantitative determination of high sensitive CRP in human serum. The laboratory tests and procedures were done under direct and close supervision of the specialized medical staff at the Teaching Laboratory unit of Baghdad Medical city.



Figure 1: Blood sample before centrifuging

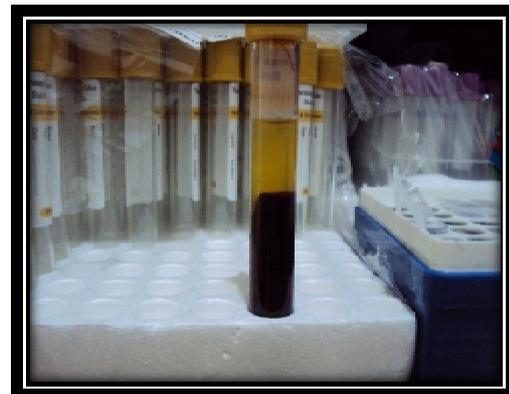


Figure 2: Blood sample after centrifuging surgical procedure

All the surgical procedures were performed by the same operator. The surgical techniques were done under local anesthesia of lidocaine hydrochloride 2% local anesthetic 2.2 ml with adrenaline 1:80000. In case of removal of impacted lower third molar, a 2-sided flap was used, through buccal approach.



Figure 3: Periapical radiograph for impacted mandibular 3rd molar

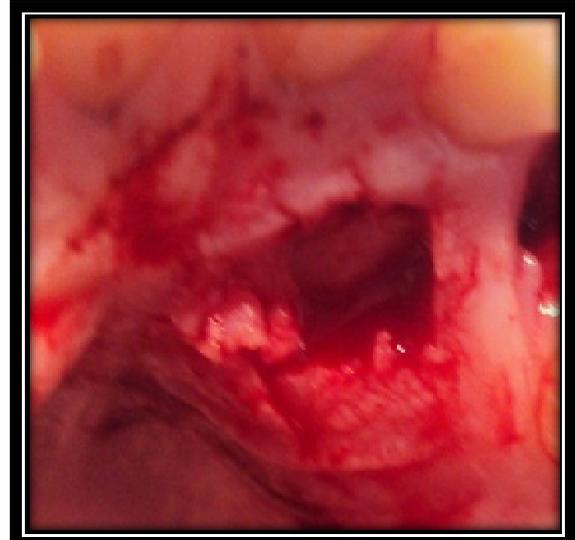


Figure 6: Maxillary canine exposure

In case of apicoectomy, a three sided flap was used through labial approach.



Figure 4: Impacted mandibular 3rd molar removed from the socket

In case of exposure of impacted canine, an envelope flap was used through palatal approach.



Figure 7: Periapical radiograph for maxillary left central incisor with periapical lesion



Figure 5: Periapical radiograph for impacted maxillary canine



Figure 8: Periapical surgery for maxillary left central incisor with periapical lesion

Then the flap was sutured by using 3/0 black silk suture using simple interrupted technique. The length of the surgical procedure was measured from the starting of the incision to the finishing of the flap suturing.

Laboratory procedure

Microtiterstrips coated with anti-CRP antibody are incubated with diluted standard sera and patient samples. During this incubation step CRP is bound specifically to the wells (round mixing containers of the high sensitive CRP kit). After removal of the unbound serum proteins by a washing procedure, the antigen-antibody complex in each well is detected with specific peroxidase conjugated antibodies. After removal of the unbound conjugate, the strips are incubated with a chromogen solution containing tetramethylbenzidin and hydrogen peroxide, a blue color develops in proportion to the amount of immunocomplex bound to the wells of the strips. The enzymatic reaction is stopped by the addition of 2 N H₂SO₄ and the absorbance values at 450 nm are determined.

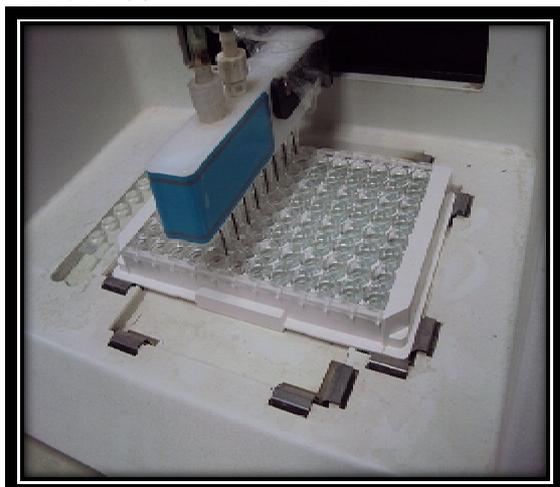


Figure 9: Microplate ELIZA washer device



Figure 10: Microplate ELIZA reader device

The data were analyzed and processed using SPSS 17 (statistical package for social science). Descriptive and inferential analyses were used to analyze the relation among the three groups including ANOVA test (Two way), Chi-Square Test, Wilcoxon Signed Ranks Test, Mann-Whitney Test, Kruskal-Wallis Test.

The level of significance was accepted at P-value < 0.05, highly significance at P-value < 0.01, and non-significance at P-value > 0.05.

RESULTS

From (48) patients who had participated in this study, (8) were excluded from this study either because they did not come back again after the surgical procedure or because they did not follow the post-operative instructions properly.

A final number of 40 patients have been accepted for statistical analysis. They were divided into twenty one males (52.5%) and nineteen females (47.5%). They have been arranged into three groups as Apicoectomy group (Ap.) that included (11) patients, Impaction group (Im.) that included (20) patients, and Exposure group (Ex.) that included (9) patients.

The mean value of the levels of CRP at the day of operation was (2.40 mg/L) and the standard deviation was (± 2.16) while the mean value of the levels of CRP at the 2nd day of

operation was (8.48 mg/L) and the standard deviation was (± 6.20).

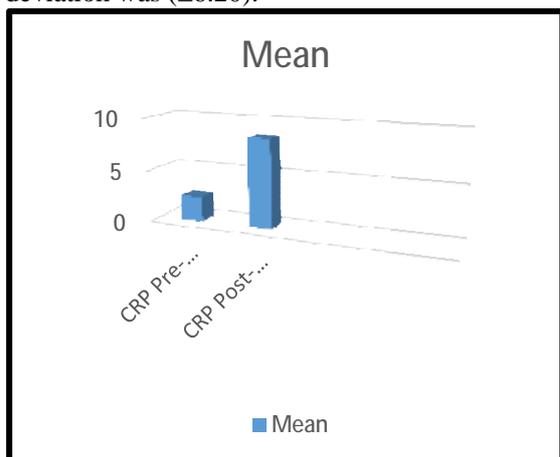


Figure 11: pre- and post-operative CRP levels

The level of CRP at the 2nd day was higher than the level of CRP at the day of surgery (pre-operatively) in all types of operations which indicated that there is a difference in the CRP levels and it is highly significant (P-value=0.000) and there was a significant difference in CRP levels in each type of operation which were (P-value=0.003, P-value=0.000, and P-value=0.008) for apicoectomy, impaction, and exposure respectively.

The level of CRP at the 2nd day of surgery in each type of operation revealed that the mean value and the standard deviation were as follow for Apicoectomy (13.02 mg/L and ± 6.39); for Impaction (8.98 mg/L and ± 4.83); and for Exposure (1.82 mg/L and ± 1.59) with mean rank (28.55, 22.65, 5.89) for Apicoectomy, Impaction, and Exposure respectively, and there was a high significant difference between apicoectomy, impaction, and exposure operations in the level of CRP at day two post-operatively (P-value=0.000).

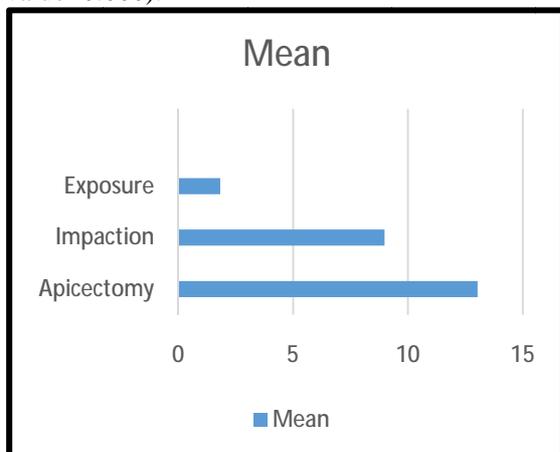


Figure 12: Level of CRP at 2nd day of surgery

There was a high significant correlation between the time of surgery (length of the surgical procedure) and the CRP level at the 2nd day of operation (correlation coefficient=0.560 and P value=0.000).

The mean value and the standard deviation of the time of surgery were as follow for Apicoectomy (20.90min. and ± 9.62); for Impaction (20.70 min and ± 12.15); and for Exposure (19.55 min and ± 14.08) with mean rank (30.64, 19.03, 11.39) for Apicoectomy, Impaction, and Exposure respectively and there was a significant difference among apicoectomy, impaction, and exposure operations in the time of operation (P-value=0.001).

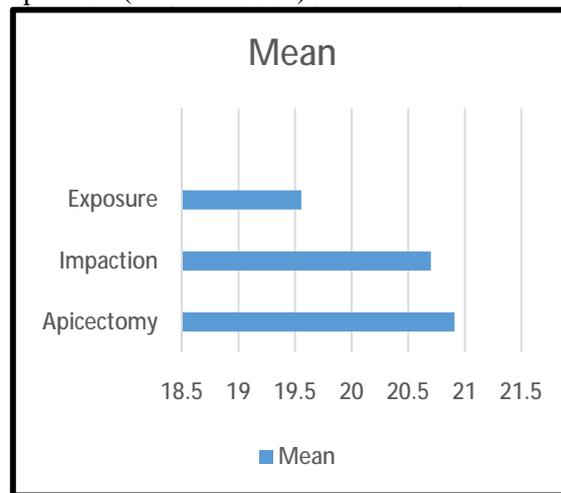


Figure 13: time of surgery in each operation type

DISCUSSION

The statistical analysis revealed that the levels of CRP post-operatively were higher than pre-operatively in all types of operations and there was a high significant difference among them which indicated that CRP level increase after surgical intervention in all types of operations. This is in agreement with Chander et al⁽¹²⁾, Desai⁽¹³⁾ and Hao Shen et al⁽¹⁴⁾ all those researchers maintained that after surgical intervention, the level of CRP raised in all patients.

These findings can be explained by the fact that the surgical trauma create a unique metabolic reaction involving a raise in the circulating stress hormones and a raise in the production and secretion of different acute-phase proteins like the CRP which is located in the plasma of the blood and its concentration increase in response to inflammatory condition because of it is an acute phase protein. The statistical analysis showed that there is a high significant difference among Apicoectomy, Impaction, and Exposure operations in the level of CRP at day two postoperatively and the highest value was found

in Apicoectomy and the lowest value was found in Exposure. This may be attributed to the more degree of tissue damage and trauma as a result of the more surgical handling of the soft and hard tissues in Apicoectomy procedures as compared to Exposure procedures or may be due to the longer operation time in Apicoectomy compared to Exposure operation. The statistical analysis showed that there is high significant relationship between the time of operation and the level of CRP at the 2nd day of operation with all types of operations, and there was highly significant difference among Apicoectomy, Impaction, and Exposure operations in the time consumed in these operations.

These findings are in agreement with Maria et al⁽¹⁵⁾ and Sanchis et al⁽¹⁶⁾ who maintained that the postoperative tissue reactions following oral surgery have been commonly related with the length of the surgical intervention. These findings can be explained by the fact that the more operation time means more tissue damage which lead to more elevation in CRP levels. Ohzato et al⁽¹⁷⁾ showed that the magnitude of CRP reaction change instantly with the sharpness of tissue destruction, kind of inflammatory stimulation and type of tissue involved in the condition. Furthermore the longest time was found in Apicoectomy and the shortest time was found in Exposure.

In conclusion this study showed that there was significant increase in the level of high sensitive CRP in the blood of patients after all types of surgical intervention when compared with pre-operative levels. And there were significant differences in the mean levels of high sensitive CRP among different surgical procedures (Apicoectomy, Impaction, and Exposure). This research gives an idea that an inflammatory process develops after oral surgical interventions so we recommend the use of anti-inflammatory agents after these surgical procedures to reduce the inflammatory process. The level of high sensitive CRP in the patient's serum appear to be positively correlated with the time of the surgical procedure which increase in the prolonged procedures.

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