

C-reactive protein estimation: A quantitative analysis for three nonsteroidal anti-inflammatory drugs: A randomized control trial

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ABSTRACT

Context: C-reactive protein (CRP) estimation for quantitative analysis to assess anti-inflammatory action of nonsteroidal anti-inflammatory drugs (NSAIDs) after surgery in maxillofacial surgery.

Aims: This study was to evaluate the efficacy of CRP as a quantitative analysis for objective assessment of efficacy of three NSAIDs in postoperative inflammation and pain control.

Settings and Design: The parallel study group design of randomization was done. Totally 60 patients were divided into three groups. CRP was evaluated at baseline and postoperatively (immediate and 72 h) after surgical removal of impacted lower third molar. The respective group received the drugs by random coding postoperatively.

Subjects and Methods: The assessment of pain control and inflammation using NSAIDs postoperatively after surgical removal of impacted lower third molar was qualitatively and quantitatively assessed with CRP levels. The blood sample of the patient was assessed immediate postoperatively and after 72 h. The visual analog scale (VAS) was used for assessment of pain and its correlation with CRP levels.

Statistical Analysis: Comparison of difference in levels of CRP levels had $P < 0.05$ with immediate postoperative and baseline levels. The duration of surgery with association of CRP levels $P = 0.425$ which was nonsignificant. The pain score was increased with mefenamic acid ($P = 0.003$), which was significant on VAS.

Results: Diclofenac had the best anti-inflammatory action. There was a significant increase in CRP levels in immediate postoperative values and 72 h. CRP test proved to be a useful indicator as a quantitative assessment tool for monitoring postsurgical inflammation and therapeutic effects of various anti-inflammatory drugs.

Conclusions: CRP test is a useful indicator for quantitative assessment for comparative evaluation of NSAIDs.

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The removal of impacted third molar is one of the most common surgeries performed in oral and maxillofacial surgery field. The removal initiates an acute inflammatory response in all patients immediately, which manifests

clinically as edema, pain, and trismus. The tissue damage, which is associated with surgical manipulation of hard and soft tissues, initiates the synthesis or release of numerous endogenous pro-inflammatory compounds. Chemical mediators act together or in sequence to amplify the inflammatory response. Chemical mediators can stimulate the release of mediators by target cells themselves.^[1]

Macrophages activated at the site of tissue injury produce interleukin (IL), which induces hepatocyte to synthesize acute phase proteins (APPs). C-reactive protein (CRP), an acute-phase reactant, was discovered in the serum of patients with pneumonia by Tillett and Francis in 1930.^[2] Synthesis of APP in hepatocyte is regulated by three main inflammatory cytokines such as IL-1, IL-6 and tumor necrosis factor produced by immunocompetent cells.^[3] Some of the important APPs are CRP, haptoglobin, transferrin,

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alpha-1 Antitrypsin and ceruloplasmin.^[4] The inflammatory response is immunosuppressive, promotes phagocytosis, inhibits platelets and activates complement.

Out of various APPs CRP is very consistent in response. Therefore, it is the most satisfactory single screening test for an acute phase reactant and a useful marker for the amount of tissue injury and inflammation.^[3] Serum CRP begins to rise 4–12 h postoperatively, reaches the peak level after 24–72 h. The normal tendency of CRP is to decrease after 72 h and return to normal after 2 weeks.^[4]

The sensory signals from the peripheral site of surgical injury due to trauma induced release of algogenic substances such as histamine, serotonin, prostaglandins, kinins and substance *P* are responsible for postoperative pain hypersensitivity. Nonsteroidal anti-inflammatory drugs (NSAIDs) have been proved effective for controlling postoperative pain and should be regarded as the basic treatment. Most NSAIDs are thought to act primarily by inhibiting the enzyme cyclooxygenase (COX), thereby preventing local synthesis of prostaglandins from arachidonic acid in the inflamed tissues. NSAIDs are commonly prescribed to alleviate pain induced by inflammation.^[3]

The main influence on the concentration of APPs during the postoperative period depends on the degree of tissue damage, an inflammatory reaction associated with repair and the regeneration processes which act to restore the integrity of the injured tissues. There is a direct positive correlation between the concentrations of APPs, especially of CRP and the severity of inflammation.^[4] The immediate postoperative sequelae of pain, buccal swelling and trismus provide a useful clinical model for evaluating the efficacy of analgesics and anti-inflammatory drugs.^[5]

A number of studies have been conducted earlier to assess the degree of inflammation after the removal of impacted third molar. Also, attempts have been made using different anti-inflammatory drugs keeping postoperative symptoms such as pain and swelling as parameters to check their efficacy. These parameters are subjective, and they lacked the sensitivity.

This study aims at the objective assessment of the surgical inflammation after removal of impacted third molar with the reliable CRP levels and hence to quantify the efficacy of the three NSAIDs.

SUBJECTS AND METHODS

A double-blind randomized study was carried out involving 60 patients scheduled for the surgical removal of an impacted mandibular third molar at the Department of Oral and Maxillofacial Surgery from September 2008 to December 2010 at Dr. D. Y. Patil Dental College and Hospital, Pimpri, Pune.

Sample size

A total of 60 patients fulfilling the inclusion criteria listed below were included in the study. A pilot study was conducted with 12 patients 4 in each group with 95% confidence level and hence the sample size of 60 was derived. The institutional review board with ethical clearance was taken before the study started. The inclusion criteria were patients with negative qualitative CRP test and healthy young adults of both genders belonging to age group of 20–30 years. A thorough clinical history was recorded followed by detailed clinical examination. Preoperative radiological examination consisted of intra-oral periapical radiograph and orthopantomogram. The laboratory investigations were done preoperatively were complete hemogram, bleeding time, clotting time and qualitative analysis of CRP. The written informed consent was obtained for the study and surgery.

A venous blood sample of approximately 2 ml was collected to qualitatively assess the CRP levels before surgery. The blood sample was transferred to plain sterile glass test tubes and then centrifuged at 2000 rpm for three to 5 min. The top layer of clear serum in the tube was separated with the help of a micropipette. One drop of serum was mixed with CRP latex reagent on a glass slide. Elevated CRP concentration of above 6 mg/l leads to visible agglutination of the latex particles. Patients with positive agglutination were excluded from the study as it indicated preexisting inflammation [Figure 1]. The surgical removal of impacted mandibular third molar was performed under local anesthesia. The time of start of surgery from administration of local anesthetic to suturing of the wound was noted. A second blood sample for quantitative estimation was collected immediately after surgery and that CRP level was considered as base-line value. A quantitative turbidimetric test kit of Spinreact company was used for the measurement of CRP in patient's serum. One of the three NSAIDs was dispensed to the patient postoperatively by lottery method and the postoperative instructions were given. The three NSAIDs, which were used in the study, were mefenamic acid 500 mg (Svizera), ibuprofen 400 mg (Abott), diclofenac 50 mg (Sunpharma). The 60 patients were divided into three groups of 20 each, according to the use of the above-mentioned NSAIDs. The

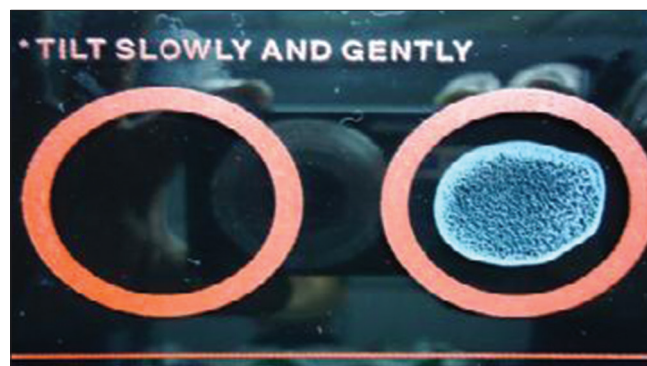


Figure 1: Agglutination visible macroscopically

allocation ratio was 1:1:1 with parallel study group design of randomized control trial was adapted. They were prescribed in a double-blinded manner where the principal investigator and patient were blinded. The bias was reduced by allocation concealment plain pouches for drugs, which were dispensed by third person who coded it. The drug diclofenac was used as benchmark control. No analgesics or sedatives were administered before surgery. The patient was advised to consume one tablet 8 hourly. A third blood sample was collected for CRP estimation after 72 h. The postoperative edema and trismus was evaluated. On the third day after 72 h, patient was given visual analog scale (VAS) to assess pain on the day of completion of the analgesic regimen. This scale was another parameter to analyze the clinical symptoms and thus compared with CRP levels. The data was analyzed by using statistical computer software SPSS 11.0 (Statistical package for social sciences 11.0 version of SPSS Inc.). The means and standard deviations were estimated from the samples for each study group. The mean values were compared by using one-way analysis of variance (ANOVA).

RESULTS

The patients enrolled in this study were 60 healthy young adults (31 men, 21 women) requiring removal of mandibular third molar with no preexisting inflammatory condition.

A value of $P < 0.05$ significant (S) was considered to indicate significant difference in statistical analysis, $P < 0.001$ highly significant (HS) considered highly significant and $P > 0.05$ not significant. The null hypothesis was that there will be no difference in CRP levels in comparison with efficacy of NSAIDs.

Study population demographics

The demographics of the study population with their gender distribution were done. There was no significant difference among the test groups with respect to age ($P = 0.929$) or gender ($P = 0.215$)

- Association of CRP levels with duration of surgery
As all the surgeries performed were completed with the mean duration of surgery taken as 45 min. The rise in CRP levels immediately after completion of surgery was compared in terms of
 - Surgery completed in ≤ 45 min
 - Surgery completed in ≥ 45 min.
 The comparison was done using unpaired t -test ($t = 0.804$) and statistical results were nonsignificant since $P = 0.425$. Thus, it could be concluded that there was no significant correlation between the CRP levels and duration of surgery since ANOVA ($F = 0.170$) and $P = 0.844$.
- Comparison of the baseline CRP levels
There was no significant difference in the values ($F = 0.494$ and $P = 0.163$) in the mean CRP level at baseline (immediate postoperative). From this, it could

be interpreted that the degree of inflammation just immediately after surgery was similar in the test groups.

- Comparison of difference in CRP levels between immediate postoperative value and the value after 72 h $P = 0.002$ shows a significant difference between three test groups. Thus, there was no significant rise in CRP levels from their baseline values. This indicates that the degree of inflammation increased in postoperative period in spite of anti-inflammatory regime [Table 1].
- Comparison of percentage increase of CRP in test groups [Table 2 and Figure 2]

On the comparison of CRP levels in test groups, significant difference $P = 0.025$ was noted. Mefenamic group showed the highest percentage of increase in CRP of 514% and diclofenac the lowest of 364%. Ibuprofen showed 406% increase.

From this it can be interpreted that

- Mefenamic group with the highest increase in CRP is the weakest anti-inflammatory drug among the three
- Diclofenac group is the most effective in controlling postoperative inflammation and can be considered as best drug amongst the three. Although the ibuprofen group showed intermediate increase, it is close to diclofenac group and can be considered as second choice of drug for postoperative management.
- Comparison of pain score on VAS Table 3

Table 1: Comparison of difference in CRP levels immediate postoperative and 72 h later

Groups	n	Difference in CRP		ANOVA F	P
		Mean	SD		
Diclofenac	20	16.305	3.977	6.927	0.002 sig
Ibuprofen	20	17.116	7.545		
Mefenemic	20	22.962	6.440		

SD=Standard deviation, CRP=C-reactive protein, ANOVA=Analysis of variance, Sig=Significant

Table 2: Numerical representation of the graph of Figure 2

Drugs	Mean
Diclofenac	364.608
Ibuprofen	405.816
Mefenemic	514.679

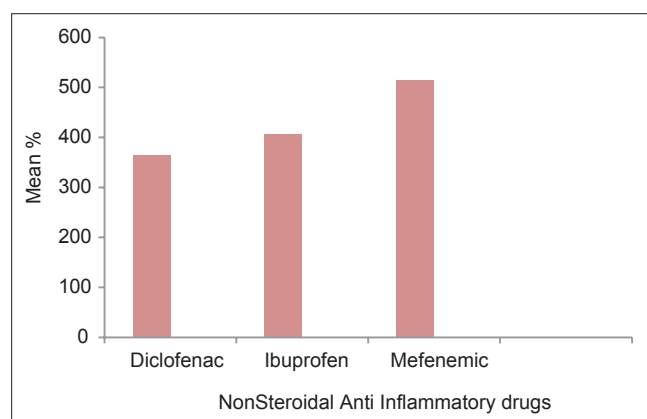


Figure 2: Comparison of percentage increase in C-reactive protein

Table 3: Comparison of pain score on VAS

Groups	n	Pain score		Kruskal-Wallis test	P
		Mean	SD		
Diclofenac	20	2.65	0.489	11.69	0.003 sig
Ibuprofen	20	2.70	0.470		
Mefenemic	20	3.20	0.523		

SD=Standard deviation, VAS=Visual analogue scale, Sig=Significant

$P = 0.003$ shows a significant increase in pain scores between three test groups and by Kruskal–Wallis test = 11.69. From this we concluded that the maximum severity of pain felt was experienced with mefenamic group followed by ibuprofen and then diclofenac group.

- Association of VAS and CRP levels after 72 h
When pain score on VAS was compared with CRP levels, it showed a direct correlation between the two parameters. Thus, the null hypothesis was rejected hence the CRP is an ideal APP for objectively quantifying postsurgical inflammation.

DISCUSSION

The most common complaint after removal of mandibular third molar is pain and swelling. There has been no authentic method for measuring these postoperative inflammatory symptoms published in the literature. The results were based on clinical assessment in most of the published literature. The investigators utilized subjective measurements, which were of doubtful statistical significance. In the past attempts, were made to measure the swelling of cheek by means of tape and photographic analysis, which are rather variable.^[6]

At the same time, investigators suggested various medicaments which claimed to reduce postoperative inflammation. A vast volume of work has been carried out concerning the efficacy of these medicaments such as anti-inflammatory compounds, proteolytic enzymes, and steroid preparations. Food and Drug Administration of United States of America focused its attention on questionability of these drugs. The emphasis was given on objective series of methods for their authenticity. Unfortunately, all the evaluations were based on subjective methods, which made statistical analysis impossible.^[7] For a reliable investigation of different factors involved in postoperative inflammation, a simple, objective method is necessary. CRP is a sensitive indicator of low grade and acute inflammatory reactions, which fulfills this requirement.

C-reactive protein as an inflammatory marker

Stahl^[8] measured daily serum APPs in general surgical procedures and found CRP as a useful marker of amount of tissue injury among all APPs.

Iizuka and Lindqvist^[9] observed that CRP levels always increased after surgery and found CRP test more appropriate for early detection of inflammation than Erythrocyte Sedimentation Rate. Freitas^[10] *et al.* studied

anti-inflammatory effects of nonsurgical lasers using CRP as an inflammatory marker. Therefore in our study, CRP test was used as a marker for assessing postoperative inflammation in mandibular third molar surgery.

Age as criterion for patient selection

Tillet and Francis^[11] discovered that CRP was precipitated from the sera of acutely ill-patients and not found in sera of normal healthy patients. In our study, preoperative qualitative analysis of CRP was a marker for this evaluation. Palosuo^[12] *et al.* studied the significance of CRP levels in different population and concluded that raised levels of CRP were found 3 times more frequently in random middle-aged population. Therefore in our study, normal healthy individuals with an age group of 20–30 years were included.

C-reactive protein level – A criterion for inclusion of subjects in this study

Gaweda^[13] discovered that CRP was present in trace amounts in the sera of normal healthy subjects. Beck and Offenbacher^[14] regarded CRP concentration exceeding 10 mg/l as an indicator of inflammatory disease. In our study, subjects were included by screening them for CRP levels by qualitative method. As per the directions of manual of laboratory kit (RHELAX CRP latex reagent), elevated CRP concentration (≥ 6 mg/l) leads to visible agglutination which concludes that individual has existing inflammation. Thus, patients with a negative agglutination were included in the study.

Timings of C-reactive protein estimation

Ohzato *et al.*^[15] *et al.* examined CRP in 71 patients and found that CRP started increasing 12 h postoperatively and was at its maximum at 48–72 h. Giannoudis PV^[16] *et al.* monitored blood samples of trauma victims at a time interval of 24 h, 3, 5 and 7 days. He also had similar observations as that of Ohzato *et al.* El-Sharawy^[17] *et al.* in their study determined CRP levels immediately after third molar surgery which was considered as a baseline value. This study also concluded that CRP level had increased to its maximum after 72 h and returned to the normal value within a week. Therefore, in our study CRP levels immediately after surgery were considered as a baseline value and were compared with CRP levels at 72 h.

Choice of nonsteroidal anti-inflammatory drugs

Despite more than 100 years of clinical experience with the prototypic NSAID aspirin, controversy persists over the mechanisms of action of these drugs. A major hypothesis familiar to many clinicians is that NSAIDs produce analgesic and anti-inflammatory actions by inhibiting cyclo-oxygenase enzyme, thereby reducing the synthesis of arachidonic acid metabolites such as prostaglandins and thromboxanes. Prostaglandins play a key role in the development of inflammation and pain. Therefore, it is predictable that the NSAIDs have clinical efficacy for reducing acute pain and inflammation. In support of this point, numerous double-blind placebo-controlled clinical trials have demonstrated that

the NSAIDs are effective for reducing pain due to surgical, periodontal and endodontic procedures. Moreover, systematic reviews of these studies support the clinical recommendation that NSAIDs should be the analgesics of choice in patients who can tolerate this class of drugs.^[1] Many NSAIDs including ibuprofen, aspirin, diclofenac, mefenamic acid, ketoprofen, ketorolac, and flurbiprofen – have been shown to produce significant reductions in pain. In our study, mefenamic acid (500 mg), ibuprofen (400 mg), diclofenac (50 mg) were compared for their efficacy.

Monitoring the drug therapy by C-reactive protein test

Halevy *et al.*^[4] proposed CRP as biochemical evidence supporting his clinical observations in operated cases. Freitas^[10] *et al.* studied anti-inflammatory effects of lasers after third molar surgeries using CRP test. Cole *et al.*^[18] in his study concluded that CRP test has a role in monitoring the treatment response. Based on above findings we used CRP test as a parameter to assess the degree of inflammation, as well as the effects of NSAIDs in controlling postoperative inflammation. In our study, significant increase in CRP level was observed in all the groups in spite of the administration of anti-inflammatory drugs. Mefenamic group showed the highest percentage of increase in CRP levels (i.e. 514%) followed by Ibuprofen (406%) and diclofenac group (364%). Thus, mefenamic group was the weakest among the groups. Pajarola^[19] *et al.* in their study compared the effect of selective COX-2 inhibitors with NSAID after third molar extraction in 30 subjects and found increase in pain curve with mefenamic acid, which was reported similar in our study. Brooks *et al.*^[20] reported in their study that the efficacy of diclofenac was due to a smaller size, tolerability as a NSAID. Collins^[21] *et al.* systematically reviewed to compare the relative efficacy of two common analgesics, ibuprofen and diclofenac, in postoperative pain and found no significant difference between the drugs. The drugs used in our study showed no side effects, as probably the drug regime was for a short duration.

Association of visual analogue scale and C-reactive protein levels after 72 h

When literature was reviewed, no such association was documented in any of the previous studies. In this study, we made a humble attempt to correlate an objective parameter with a subjective one that is CRP levels with VAS.

In our study, we found a direct correlation between these two parameters.

CONCLUSION

The randomized control trial showed a significant increase in CRP levels at the end of 72 h in all three test groups. The diclofenac had the best anti-inflammatory action in comparison to ibuprofen and mefenamic acid. CRP test is a useful indicator to monitor postsurgical inflammation and therapeutic effects

of various anti-inflammatory drugs in an objective manner. Further long-term longitudinal studies are required to see its utility in other oral and maxillofacial surgeries.

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