Research Article

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Estimation of C-Reactive Protein as an Inflammatory Marker in the Infection of Odontogenic Origin: A Cross Sectional Study

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ABSTRACT

Aim: The aim of the study to determine the level of serum CRP by quantitative method pre-operatively and post-operatively and also need to determine the need of continuation of the antibiotics therapy and monitor the infection of odontogenic origin.

Material and methods: Patient's blood samples (N=35) were collected for the assessment of Serum CRP and routine blood investigations on the day the procedure was started, post operatively after antibiotic therapy. Serum CRP is again measured by quantitative method.

Results: There was onset of disease 32 (91.4%) of cases were acute and the level of Serum CRP was positive (more than 0.6 mg/dl) in 28 cases (80%) of patients pre operatively. In 7 cases (Serum CRP level was negative) found no sign of infection or inflammation after 1 week. The dentoalveolar abscess was most commonly involved in 9 cases followed by periapical abscess 7 cases and buccal space infection 4 cases.

Conclusion: The findings of this prospective analysis indicate that Serum CRP help in diagnosis and monitoring of the disease and helps in prognosis and assessment of level of serum CRP is much more sensitive in cases of infections of odontogenic origin.

Keywords: C Reactive protein, Inflammatory marker, Odontogenic infections.

INTRODUCTION

Patients with fascial space infections of odontogenic origin are at utmost risk for life-threatening situations due to anatomical connectivity of potential spaces to one another. Lethal complications may become inevitable making vigilant scrutiny and monitoring of such patients a necessity. Although conventional measures to estimate infections such as White Blood Count (WBC) and Erythrocyte Sedimentation Rate (ESR) values are valuable in determining state of patient at testing time, the predictability of these is worth limited. The desirability of serum derived surrogate predictor behavior and outcome cannot be under estimated, arousing interest in identifying substances which could function as prospective monitor of disease progression. Thus, various inflammatory markers came into existence [1].

C reactive protein is one of the most dynamic acute phase proteins among the pentraxin protein family. Its serum concentration can increase 1000 fold or more in various stimuli associated with infections and other types of tissue injuries. Similar to the application of erythrocyte sedimentation rate

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(ESR) and white blood cell (WBC) counts, testing for CRP is most commonly performed to indicate the presence of acute inflammation and to monitor the development of postoperative infections. CRP elevates significantly within 4 to 6 hours, peaks in 24 to 48 hours after the acute infection of tissue injury occurs, and falls rapidly after the inflammation resolves. Rapid rise and fall of CRP with inflammatory process makes it much more sensitive indicator for infection and inflammation compared to ESR and WBC count. The close association of serum CRP concentration with the nature and intensity of the activating stimulus has allowed CRP to be used to discriminate bacterial from viral infections, and gauge therapeutic responses for inflammatory diseases [2]. Serum CRP can rise up to 100 to 200 mg/l in bacterial infection and can slightly rise (20 – 40 mg/l) in viral infection. Elevated CRP is found in CSF of bacterial meningitis while in case of viral meningitis CRP is not found to be elevated [3].

C-reactive protein (CRP) is a highly conserved plasma protein that participates in the systemic response to inflammation [4]. It acts as a pattern recognition molecule typically exposed during cell death or found on the surfaces of pathogens [5]. Thus CRP contributes to host defense and plays a crucial role in the first line of innate host defense.

Synthesis of CRP rapidly and dramatically increases within hours after tissue injury or infection, making it useful in determining disease progress or the effectiveness of treatment [4]. if the patient recovered, the substance is again undetectable. It has also been used to gauge the inflammatory response in chronic diseases, such as vasculitis and rheumatoid arthritis, and found in the blood of patients with febrile diseases [5].

An association between minor CRP elevation and future major cardiovascular events has been recognized, leading to a recent recommendation by the Centers for Disease Control and the American Heart Association that patients at risk for heart disease might benefit from measurement of CRP [6]. The risk factors for cardiovascular disease such as age, obesity, smoking, and diet are all factors that would increase CRP levels in the body.

CRP was found to be closely involved in the process of atherosclerotic plaque development by inducing adhesion molecule expression in human endothelial cells, activating vascular smooth muscle cells that lead to the induction of monocyte chemo attractant peptide, and opsonizing low density lipoprotein to facilitate its uptake by macrophages [2].

Serum CRP is nearly absent in healthy individuals and increases significantly when tissue damage occurs during infection, tissue injuries, or inflammation. Cytokines produced and released during tissue damage and inflammation, specifically interleukin-6 and TNF–α (tumor necrosis factor α) induce the production of CRP in hepatocytes. CRP production is proportional to the severity of tissue damage [7].

C-reactive protein has long been recognized as an innate opsonin, that is, a protein that recognizes microbes and promotes their uptake by phagocytic cells. CRP also recognizes apoptotic and necrotic host cells and plays an important role in their clearance. This would contribute to the restitution of normal function and structure of injured tissues [2].

Determination of CRP is used to monitor the therapeutic efficacy of different treatment regimens on infection. Its application in monitoring post operative infections is also one of the subjects of interest. CRP testing may be also useful tool in monitoring the recovery process after third molar surgery and identifying patients who need antibiotic therapy [2].

In our study conducted we are determining the level of serum CRP by quantitative method pre-operatively and post-operatively and also need to determine the need of continuation of the antibiotics therapy and monitor the infection of odontogenic origin.

MATERIAL AND METHODS

A prospective study of serum C-reactive protein estimation was conducted in patients with odontogenic infection at Department of Oral and Maxillofacial Surgery, Pacific Dental College and Hospital, Udaipur, Rajasthan. The database collection included variables like age, sex, medical

history, site and number of spaces involved, tooth which is involved, intra oral periapical radiograph of involved tooth or orthopentogram. Patient's blood samples were collected for the assessment of Serum CRP and routine blood investigations if required, on the day the procedure was started (antibiotic therapy/ extraction/ incision and drainage), post operatively (1 week to 2 week) after antibiotic therapy. Serum CRP is again measured by quantitative method. Then results were compared between the result at time of procedure and the serum collected post-operatively.

Pacific Academic of Higher Education and Research university ethical committee provided the ethical approval for this study. Informed consent was obtained from all study subjects by ensuring confidentiality and explaining the risks-benefits involved.

Inclusion Criteria

1) Patient with odontogenic infection.
2) Patients age ranging from 17 to 60 years.

Exclusion Criteria

1) Patients with systemic disease.
2) Patients with Hormone therapy, pregnancy, contraceptive pills, intrauterine device.
3) Patients with previous antibiotics therapy.

Procedure

Patients diagnosed with infection of odontogenic origin who visited Pacific dental college and hospital is included in the study with age ranging from 17 to 60 years. Total 35 patients were according to inclusion and exclusion criteria from January 2013 to January 2014. Blood sample will be drawn from anticuboidal fossa and laboratory test will be carried out for the estimation of serum CRP and other routine blood investigations. Level of CRP is estimated at the time patient reported with the infection and again estimated after a week or two weeks. Normal CRP value was less than 0.60 mg/dl.

Evaluation Criteria

1) Preoperative assessment of name, age, gender, medical history, site and number of spaces involved, tooth which is involved, intraoral periapical radiograph of the involved tooth or orthopentogram or both. Collection of blood sample for the estimation of serum CRP level and routine blood investigations.

2) Intraoperative assessment with treatment given to the patient::-

a) Tooth extraction,
b) Surgical procedure - incision and drainage.

3) Post operative clinical evaluation and by estimation of Serum CRP for comparison after a week or two weeks.

Interpretation of the Result

Positive result - The concentration of CRP more than 0.60 mg/dl
Negative result - The concentration of CRP lower than 0.60 mg/dl

Quantitative Test

Turbidimetric immunoassay for determination of C-reactive protein.

Reagent

1. Turbilyte CRP activation buffer (R1)

2. Turbilyte latex reagent (ready to use uniform suspension of polystyrene latex particules coated with anti CRP antibody) (R2)

3. Turbilyte calibrator (lyophilized preparation of serum equivalent to the stated amount of CRP on mg/dl basis when hydrated approximately. The caliber is traceable to the W.H.O. international reference standard (85/506) for human C-reactive protein).

Reagent Storage and Stability

1. Store the reagent at 2 to 8 degree Celsius and do not freeze.
2. The shelf life of reagent, activation buffer and calibrator is as per expiry date mention on vial.
3. The working reagent for tubilyte CRP can be prepared by mixing R2 and R1 in ratio of 1/10.
4. The mix stability of working reagent R1+R2 is 7 days when stored at 2 degree Celsius.

Principle

Turbilyte CRP is a turbidimetric immunoassay for the determination of C-reactive protein in human serum and is based on the principal of agglutination reaction the best specimen is mixed with the activation buffer R1. Turbilyte CRP latex reagent R2 and allowed to react. Presence of CRP in the test specimen result in the formation of an insoluble complex producing a turbidity which is measured at 546 nm wavelength. The increase in turbidity corresponds to the concentration of CRP in the test specimen.

	For calibration	For sample
R1	450 µl	450 µl
R2	50 µl	50 µl
MIX WELL AND INCUBATE FOR 5 MINUTE		
CALIBRATOR	3 µl	-
SAMPLE	-	3 µl
MIX WELL AND READ ABSORBANCE A1 TO 10 SECONDS AND A2 AT 2 MINUTES		

Specimen Collection and Preparation:

Only serum was used for testing. In case of delay in the testing, the sample was stored at 2 to 8 degree Celsius. Sample can be stored at 2 to 8 degree Celsius for 3 days provided they are not contaminated. Hemolysed, icteric or highly turbid sera was not used. Turbid or particulate serum samples were been clarified by centrifugation at 2000 rpm for 15 minute and clear supernatant serum was used for testing.

Additional Material Required:

Spectro photometer with 546 nm wavelengths filtered with cuvette mode, stop watch, well calibrated micro pipette, disposable tip, isotonic saline, particulate free distilled water, test tube rack, incubator/water bath set at 37 degree Celsius. Optically clean disposable /glass semi micro cuvettet.

Test Procedure:

Reagent and sample were bought at room temperature before use.

Assay Conditions

Wavelength - 546 nm
Reaction temperature - 37 degree Celsius
Cuvette - 1 cm path length

The tubilyte CRP calibrator must be reconstituted exactly with the standard amount of distilled water, wait for 10 minute, gently swirl the vial till the solution attains homogeneity. Once reconstituted it is ready to use for CRP calibration.

RESULTS

A total of 35 patients with infections of odontogenic origin participated in the present study and their level of serum CRP were assessed and compared between pre operatively and 1st or 2nd week post operatively. Age distribution was statistically significant as 60 % of the patient's age ranging between 18 to 30 years. There was statistically difference in case of gender distribution males were more affected 62.9% then females (Table 1). There was statistically difference in the onset of disease 32 (91.4%) of cases were acute in origin and depending on the condition 31 (88.6%) of cases were diagnosed as abscess (Table 2).

The level of Serum CRP was positive (more than 0.6 mg/dl) in 28 cases (80%) of patients pre operatively and was negative (less than 0.6mg/dl) in 7 cases (20%) of patients out of which 3 patients were diagnosed as odontogenic infection with extra oral draining sinus and other 4 cases were diagnosed as cellulitis. All the patients underwent extraction of tooth or extraction of tooth along with incision and drainage (Table 3) as per requirement on clinical assessment. The patients were followed by post operative antibiotic therapy, out of 35 cases 22 (62.9%) cases were prescribed with oral antibiotics (amoxicillin with clavulanic acid 625 mg, metrogyl 400 mg and analgesics for 5 days) and discharged same day and 13 (37.1%) cases were admitted in post operative care ward and prescribed with intravenous antibiotic (injection amoxicillin with clavulanic acid 1.2 gm, injection gentamycin 20mg, injection metrogyl 500 mg, and analgesics for 5 days).

One week post operatively 35 patients reported back for follow up evaluation. In 7 cases the pre-operative Serum CRP level was negative and based on clinical evaluation no sign of infection or inflammation was present after 1 week, Thus 2nd Serum CRP evaluation was not carried out in those 7 cases. In other 28 cases CRP levels decreased after treatment in all 28 patients. CRP was found to be within normal range in 18 (64.3%) and in 10 (35.7%) it was still above normal range but significant decrease in the value when compared to pre-operative serum CRP (Table 3).

The dentoalveolar abscess was most commonly involved in 9 (25.7 %) cases followed by periapical abscess 7 (20%), buccal space infection 4 (11.4%), submassetric space infection 3 (8.5%), ludwig's angina 2 (5.7%), submandibular and submassetric space infection 2 (5.7%), submandibular space infection 1 (2.8%), submandibular and pterygomandibular space infection 1 (2.8%), submandibular and temporal space infection 1 (2.8%), buccal and canine space infection 1 (2.8%), buccal and submandibular space infection 1 (2.8%), submassetric,submandibular and submental space infection 1 (2.8%) . (Table 4)

Table 1: Descriptive distribution among odontogenic infection patients.

		N (%)
Age (in year)	18-30	21 (60)
	31-40	8 (22.9)
	41-50	4 (11.4)
	51-60	2 (5.7)
Gender	Male	22 (62.9)
	Female	13 (37.1)
	Total	35 (100)

Table 2: Diseases data distribution among odontogenic infection patients.

		N (%)
Onset of disease	Acute	32 (91.4)
	Chronic	3 (8.6)
Condition	Abscess	31 (88.6)
	Cellulitis	4 (11.4)

Table 3: CRP count among odontogenic infection patients.

Before treatment	N (%)
Negative (< 0.60)	7 (20)
Positive (> 0.60)	28 (80)
0.60-3.06	14 (40)
3.07-5.53	7 (20)
5.54-8	4 (11.4)
8.01-10.47	3 (8.6)
after treatment (N=28)	
Negative (< 0.60)	18 (64.3)
Positive (> 0.60)	10 (35.7)
0.60-3.06	9 (32.1)
5.54-8	1 (3.6)

Table 4: Site distribution of odontogenic infection patients.

Site	N (%)
Periapical abscess	7 (20)
Dentoalvelolar abscess	9 (25.7)
Canine	1 (2.8)
Buccal	4 (11.4)
Submandibular	1 (2.8)
Submassetric	3 (8.5)
Ludwing's Angina	2 (5.7)
Submandibular + Submassetric	2 (5.7)
Submandibular + Pterygomandibular	1 (2.8)
Submandibular + Temporal	1 (2.8)
Submassetric + Submandibular + Submental	1 (2.8)
Canine + Buccal	1 (2.8)
Buccal + Submandibular	1 (2.8)
Total	35 (100)

DISCUSSION

The classic markers of infection are fever, and leucocytosis. Although most economic is to measure body temperature, body temperature is specific but not sensitive marker of infection. There is no relation between fever and severity of the disease. High fever can be associated with the minor infections and vice versa. The WBC count is also routinely performed for the detection of the infection. WBC is influenced by many non infectious markers like myocardial infarction, catecholamines, corticosteroids and acute bleeding. More over there

are some infectious diseases that characteristically progress without leucocytosis like typhoid fever, tuberculosis, mumps, measles, and chicken pox. Thus the value of leucocytosis in the diagnosis of infection is questionable [8].

Determination of serum CRP is an economical, consistent and reproducible test and is available in almost every hospital settings. The half-life of CRP in the circulation is not significantly influenced by age and gender, and its serum concentration is largely determined by the rate at which CRP is produced and released into the blood [2]. The single determinant of CRP level is its rate of synthesis, which in turn depends on the inflammatory intensity [8]. Its serum concentration can increase 1000-fold or more in response to various acute stimuli associated with infections and other types of tissue injuries [9]. CRP level rises rapidly in Gram positive and Gram negative bacterial infections [3,10,11]. There is rapid rise of CRP during preoperative period and rapid fall post operatively compared to ESR [2,12,13].

The concentration of serum CRP level is directly proportional to amount of tissue injury occurs and increases in case of trauma, infection, inflammation and malignancy [2,7,14,15,16,17]. Similarly in present study level of serum CRP was comparatively higher in more severe infections. Assessment of serum CRP level is not completely accurate test to detect infection [12]. In study conducted by Yan-Fang Ren et al [2] found positive CRP level in 75 % cases similarly in present study positive results were obtained in 80% (35) cases while 20% (7) patients there was no rise in CRP at the time of diagnosis, out of this 20% (7) patients 3 patients were diagnosed as odontogenic infection with extra oral draining sinus, the duration of the infection was chronic in nature and the other 4 cases were diagnosed as cellulitis. The reason for it could be CRP raises significantly 24 to 48 hrs after acute infection and tissue injury occurs [2]. Because of which the cellulitis cases would have resulted negative. The determination of CRP combined with a careful clinical examination is useful when the necessity of treatment with antibiotics is being considered [12]. CRP monitoring represents a possible means of stopping antibiotics safely, sparing patients from drug toxicity, probably decreasing the emergence of resistance and decreasing costs [18]. CRP also helps in differentiating viral infection from bacterial infection as there is significant increase in CRP value in case of bacterial infection compared to viral infection [19].

Apart from infection, CRP level also elevates rapidly due to traumatic injury and post operatively due to inflammatory response. In orthopedics patients undergoing total knee replacement, total hip replacement, anterior cruciate ligament reconstruction, osteosynthesis of bones with rigid or semi rigid plates, significant rise in CRP occurs post operatively [12,14,19]. Same was been observed in our present study the CRP level was decreased as compared to pre-operative CRP but was still above normal range in severe cases.

As CRP starts elevating 4th to 6th hour and rises rapidly 24th to 48th hour after acute infection and tissue injury and also falls rapidly once the infection subsides. Therefore, instead of taking single baseline value of CRP at the time of diagnosis, serial analysis of CRP at regular interval should be done during post operative follow up period to assess the effectiveness of the treatment and recovery process [12,20]. In present study we assessed serum CRP at two points, one at time of diagnosis and the second post-operatively 1st or 2nd week to access the response of treatment and antibiotics therapy.

There are certain limitations of CRP such as it does not differentiate between post operative inflammation and infection. Immediately within first few hours of tissue injury, CRP does not elevate significantly [2]. Level of CRP can be affected by medications and other factors. Hormone therapy, pregnancy, contraceptive pills, intrauterine devices can raise CRP level. Cholesterol lowering statin drugs, anti-inflammatory drugs may lower the CRP level. Sensitivity of qualitative and semi quantitative CRP test is less than high sensitivity CRP test. In present study determination of serum CRP was done by using high sensitive turbidimetric immunoassay for Serum CRP.

CONCLUSION

The findings of this prospective analysis indicate that Serum CRP help in diagnosis and monitoring of the disease and helps in prognosis

and assessment of level of serum CRP is much more sensitive in cases of infections of odontogenic origin. Serum CRP reflects the tissue injury and severity of infection. As CRP test is economical, reproducible and available in most of the hospital setting, incorporating in management of odontogenic infections helps in stopping antibiotics safely, sparing patients from drug toxicity, probably decreasing the emergence of resistance and decreasing costs. Though CRP is not 100 % accurate test, it should always been monitored along with thorough clinical examination.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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