Original Article

Significant role of serum CRP in differentiating inflammatory from non-inflammatory causes of thyrotoxicosis

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ABSTRACT

Background: C-reactive protein (CRP), which is a marker of inflammation, has not been widely studied in inflammatory thyroid disorders particularly in sub-acute thyroiditis (SAT). **Aim:** This study was aimed to find the significance of CRP level rise in patients with SAT and compare that to the rise in erythrocyte sedimentation rate (ESR), a gold standard laboratory parameter in establishing the diagnosis of SAT. **Materials and Methods:**Serum CRP levels were measured at initial presentation in 28 subjects with SAT(12 male, 16 female, age (Mean +SD) 37.96 ±8.5 years),and 19 patients with Graves' disease (2 male, 17 female, age [Mean +SD] 36.8 ±16.5 years) as controls. Erythrocyte sedimentation rate (ESR) was measured in all 28 patients with SAT by Westergrens' method. Either Tc^{99} nucleotide thyroid scan or high resolution ultrasonography (HR-USG) was performed to differentiate SAT from Graves' disease.Fine needle aspiration cytology (FNAC) of thyroid was performed selected patients. **Results:** Serum CRP level was high in 61% of SAT patients but in none of the Graves'patients. Mean (SEM) (90%CI) serum CRP level (mg/L) was also significantly higher (P <0.0004) in the SAT group [27.55 (5.76) (15.72-39.38)], than in the Graves' group [4.09 (0.12) (3.81-4.36)]. The sensitivity of serum CRP was 73.33%, specificity 53.85%, positive predictive value (PPV) 64.71%, and negative predictive value (NPV) 63.64% as compared to the sensitivity (53.57%), specificity (15.38%), PPV (57.69 %), and NPV (13.33%) of ESR. **Conclusion:** There is significantly higher rise in serum CRP level in patients with SAT is compared to patients with Graves' disease. It correlates well with the rise in ESR. Such findings of this pilot study highlight the scope of using serum CRP as a diagnostic marker of SAT specially *in situ*ations when it may be confused with Graves' disease, another common cause of thyrotoxicosis. It is logical to carry out studies to find a particular cut-off for serum CRP which can serve as an objective parameter for grading the inflammation in patients with SAT.

Keywords: C-reactive protein,Graves', sub-acute thyroiditis, thyroiditis

Introduction

The term sub-acute thyroiditis (SAT) denotes inflammation of the thyroid which is spontaneously remitting in nature.[1] Classically, affected patients show a pseudo-granulomatous pathologic appearance in the thyroid gland which on palpation is typically firm, enlarged and tender. There are also characteristic prodormal symptoms like malaise and fever.[1]There is clinical and biochemical evidence of thyrotoxicosis secondary to the release of preformed thyroid hormones from the destroyed thyrocytes.[1,2] Amongst the laboratory findings, elevated erythrocyte sedimentation rate (ESR) is invariably present, the absence of which may question a tenable diagnosis of SAT.[3] Classically erythrocyte sedimentation rate (ESR) is the most commonly used laboratory tool to detect an inflammatory syndrome.[3]However acute phase reactants are becoming useful alternatives to ESR due to significantly higher false positivity and false negativity associated with the later.[4] The acute phase response is a non-specific phenomenon occurring early in which the concentration of a number of plasma proteins is increased following most form of

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tissue injury, infection, inflammation.[5] Alteration in acute phase reactants can be a useful indicator of infection or inflammatory response when the clinical diagnosis is in doubt. Serum C reactive protein (CRP) is one such acute phase reactant which has been found to be characteristically elevated in inflammatory thyroid disorders.[6-8] Interleukin (IL)-6 has also been found to be significantly higher in amiodarone induce thyroiditis (AID), another common subtype of inflammatory thyroid disease.[7-9] Studies have demonstrated significant elevation in chronic autoimmune thyroiditis(CAT) patients with overt and subclinical hypothyroidism compared to control. [10,11]However such a rise in serum CRP issignificantly less in CAT compared to SAT.[6] Salivary CRP also rises significantly in SAT and hence has been proposed as a helpful biomarker in differentiating such an inflammatory thyroid disorder from others.[12] Thus it has been observed that rise in CRP in blood or body secretion is most striking in SAT amongst all sorts of thyroid disorders. In various other fields of medicine, serum CRP has evolved as a sensitive marker for underlying inflammatory pathology, response to therapy and prognosis of the disease process.[5,13,14] However, it is yet to be used routinely in the evaluation of inflammatory thyroid disorders and differentiating them from various other conditions having similar clinical manifestations. One of the goals of this study was to find as to whether serum CRP levels could be used to distinguish between two common causes of thyrotoxicosis.

Low Radioactive Iodine Uptake (RAIU), often <2% in 24 hours is described as important as elevated ESR in establishing a diagnosis of SAT. A similar pattern is observed with technetium (99mTC) pertechtenate in most if not all the cases of SAT.[15] Ultrasonography (USG), particularly color-flow Doppler scans in which the gland appears hypoechoeic with low to normal vascularity also plays a supporting role in the evaluation of SAT.[15] This is in contrast to increased vascularity in Graves' disease, another cause of diffuse pathology of the thyroid causing thyrotoxicosis.[16-18] Various cytopathological features of SAT include destruction of the follicular epithelium, loss of follicular integrity, patchy lesions and infiltration of mononuclear cells. The typical features include a central core of colloid surrounded by multinucleate giant cells.[19]

Initial presentation of SAT is a prodorme of generalized myalgias, low grade fever, fatigue and symptoms of upper respiratory inflammation. Pain of varying degrees in the region of thyroid gland is noted in most if not all the cases. The pain may involve either or both lobes at a time and typically radiates from the thyroid gland to the angle of the jaw and to the ear of the affected side.[5,13,14,20] Fifty percent of patients have symptoms of thyrotoxicosis. It occurs when preformed thyroid hormone-triiodothyronine (T3) and thyroxine (T4) are released from the damaged thyroid follicular cells.[21] The phase of thyrotoxicosis is usually followed by a phase of (transient) hypothyroidism with low free T4 and high thyroid stimulating hormone (TSH) levels, which may last from weeks to months. Most of the patients usually regain euthyroid status within 6-12 months. However persistent hypothyroidism is observed in 10-15% of patients.[22-25]

We hypothesize that serum CRP levels could be a predictive marker for thyroid inflammation and can help differentiate SAT, a grossly inflammatory cause of thyrotoxicosis, from Graves' disease which is non-inflammatory in nature.

Materials and Methods

Subjects

All subjects were selected from endocrine outpatient service of a multispecialty clinic. The study protocol was approved by a local human research ethics committee. Informed consent was obtained from each of the subjects prior to inclusion in the study. Subjects who had undergone radiological studies using intravenous contrast during previous three months or were taking thyroid hormone, amiodarone, lithium or had been exposed to radioactive iodine in the past were excluded from the study. Subjects having possible and probable inflammation of any organ system other than thyroid were also excluded. The study group consisted of patients with SAT who were diagnosed on the basis of clinical presentation (neck pain, high ESR, biochemical evidence of thyrotoxicosis), supported either by 99m Tc-pertechtenate thyroid scan (low uptake) or color Doppler USG(reduced flow).The control group consisted of patients with Graves' disease. Diagnosis of Graves' disease was made on the basis of clinical and biochemical evidence of thyrotoxicosis, presence of diffuse painless goiter, high antithyroid peroxidase antibody (Ab TPO) titer, and increased uptake in Tc99 thyroid scan. Anti-thyroglobulin antibody was not measured in this study.

Evaluation of thyroid function

Thyroid function was assessed by measuring serum total triiodothyronine (T3), total thyroxine (T4), free T3 (selected cases), free T4 (selected cases)and TSH, AbTPO (selected cases) by chemiluminescent assay (Immulite 1000®, Diagnostic Product Corporation, LA, CA, USA).

Isotope thyroid scan

It was performed by using isotope 99m Tc-pertechnetate. Briefly, 5 millicurie of the isotope was injected intravenously and anterior view of the thyroid was taken under a SPECT gamma camera(MPR, Model/H30000ZK, GE Medical

System, USA) using parallel collimator to include salivary gland after 20-30 minutes of injection. Normal 20 minutes uptake by the thyroid in the local population is 0.5-2.5% of the administered dose.

High resolution USG and color Doppler of thyroid

It was performed usinga machine (Vivid3, GE Medical System, USA) with multifrequency probe (7.5-12.5 Mega Hz). Utmost care was taken to document 'hypoechoic' area(s) signifying destruction due to inflammation suggesting a diagnosis of SAT. However, we did not attempt to measure the volume ofsuch areas in question due to lack of 3-D facility. Doppler probe was used to document vascularity pattern (increased, decreased on insignificantly altered).

CRP level estimation

Serum CRP level was measured by immunoturbidometric assayusingcommercial kits (Randox laboratories Ltd, Crumlin, UK) in samples collected at the initial presentation. Briefly, unknown samples were reacted with two specific antiserum (monospecific to human CRP) to form a precipitate which was measured turbidimetrically at 340nm. Assay range was 0.3-160 mg/L. Normal serum level of CRP is<5mg/L. These assays were performed independently by one of the authors (BB) who was unaware of the modality of treatment (glucocorticoid, NSAID or anti thyroid drugs) offered to the index patient.

Statistical analysis

The results were expressed as Mean+SEM (Standard Error of Mean). Continuous variables were compared by the Student's *t*-test. Comparison of the categorical variables of age, body mass index(BMI) and CRP in patients with Graves' disease and SAT using the Mann Whitney U test. Differences between groups of patients were considered significant when *P* values were<0.05. Comparisons of sensitivity and specificity were made using McNemar's test.

For the construction of receiver operating characteristics (ROC)curves, relations between sensitivity (ordinate) and specificity (abscissa) for various cut-off points were plotted. In general, a closer location of the ROC plot to the upper left corner indicates a higher diagnostic performance of the assay. The area under the ROC curve (AUC) provides an index of the overall discriminative ability of the test. Pearson's correlation coefficient assessed the importance of the different variables. Differences were considered significant if $p<0.05$. The determination of the predictive value was done by MedCalc Software.

The statistical analyses were carried out using MedCalc Software Version 11.6.1.0 (Broekstraat Mariakerke, Belgium) and R version 2.14.1 (2011-12-22) {The R Foundation for Statistical Computing (ISBN 3-900051-07-0)}.

Results

The study group consisted of 28 patients with SAT and the control group consisted of 19 patients with Graves' disease. The characteristics of the subjects in both the groups are shown in Table 1. Age and BMI did not differ significantly. The proportion of female patients were comparatively more in the control group (male:female ratio 17:2 in the Graves' group and 16:12 in the SAT group).

Serum CRP level was higher than the cut-offfor normal population in 61% of SAT patients but none of the patients with Graves' disease had a supra-normal value[Figure 1]. Serum CRP level (mg/L)(Mean{SEM}{90%CI})was also significantly higher in the SAT group i.e. 27.55(5.76) (15.72- 39.38), than in the Graves' group, i.e.4.09(0.12) (3.81-4.36)(*P*<0.0004{HS}) [Table 1].

The sensitivity of serum CRP was 73.33%, specificity 53.85%, positive predictive value (PPV) 64.71%, and negative predictive value (NPV) 63.64% as compared to the sensitivity ofESR(53.57%), specificity (15.38%), positive predictive value (PPV)(57.69 %), and negative predictive value (NPV)(13.33%).

The ROC curves of CRP passed closer to the upper left corner than that of ESR and the AUC of CRP was significantly larger than AUC of ESR (0.774 for CRP (*P*<0.0038) and 0.751 for ESR (*P*<0.0110) [Figure 2].

Table 1: Comparison of baseline characteristics and serum CRP between patients with Graves' disease and patients with sub-acute thyroiditis

	Patients with Graves' disease (*n*=19)	Patients with sub-acute thyroiditis (*n*=28)
AGE in years (mean±SD)	36.79±16.50	37.96±8.68
BMI kg. Mr^{-2} (mean±SD)	21.26±3.24	22.56±2.97
Serum CRPmg/L: Mean(SEM)(90%CI)	4.09(0.12)(3.81-4.36)	27.55(5.76)(15.72-39.38)$^{\Psi}$
Total number with serum CRP>5mg/L (%)	0(0)	17(61)
Total number with serum CRP>10mg/L (%)	0(0)	14(50)

CRP: C-reactive protein. $^{\Psi}P$ <0.0004, Compared to patients with Graves' disease

Discussion

Around 5% of patients belonging to the spectrum of clinical thyroid disorders present with painful SAT, which is a self-limited inflammatory disorder.[1] It has been known to be the most common cause of thyroid pain which follows an upper respiratory tract infection. Sub-acute thyroiditis begins with a prodrome of generalized myalgias, pharyngitis, low-grade fever, and fatigue.[1] Patients then present with fever and severe neck pain, swelling, or both.[1,2] It is well established that up to 50% of patients have symptoms of thyrotoxicosis. In most patients, thyroid function will be normal after several weeks of thyrotoxicosis, and hypothyroidism subsequently develops, lasting four to six months, as in painless sporadic thyroiditis.[1,2] Although thyroid function normalizes spontaneously in 95% of patients over a period of 6-12 months, residual hypothyroidism persists in 5% of patients. Painful SAT recurs in only about 2% of patients.[2]

We found significantly higher serum CRP level amongst SAT patients compared to Graves' patients of similar age.[8] Serum CRP level correlated well with that of ESR (r=0.647, P<0.001) which is the gold standard laboratory tool for establishing the diagnosis of SAT.[3] While 17(61%) of SAT patients had serum CRP above the biological reference range, none (0%) of Graves' disease patient had raised CRP. Another study looking at serum CRP levels in patients with various thyroid disorders found much higher rise (>10ng/dL) in SAT compared to post-partum thyroiditis and amiodarone induced thyroiditis.[6] Among the 89 patients with thyrotoxicosis (all were multinodular and nodular goiter and none with Graves' disease) in that study, non had high serum CRP level. In fact, our study is the first one to compare two different etiological groups of thyrotoxic patients, one with inflammatory and the other with non-inflammatory background using serum CRP level as a criteria.

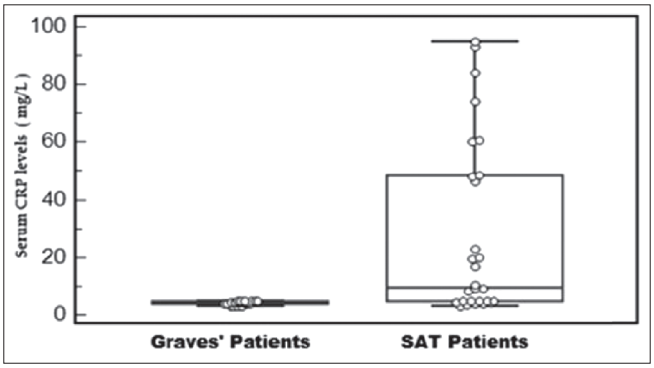

Figure 1: Box and whisker plot of serum CRP levels from minimum to maximum in Graves' disease patients and SAT patients, showing increased mean serum CRP in the later group compared to the former. Serum CRP concentrations differed significantly (P <0.0004) between the groups. The "box" in the box-and-whisker plot contains the middle half of the data points. CRP:C-reactive protein; SAT: Sub-acute thyroiditis

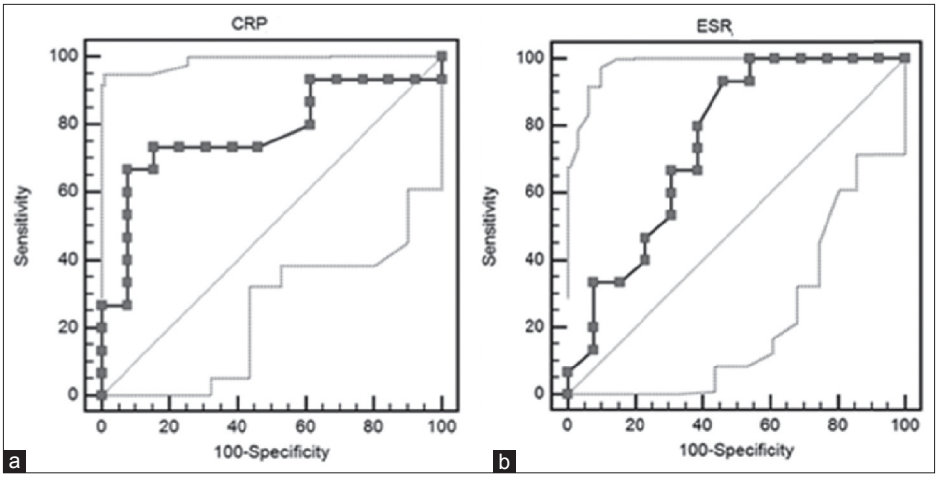

Figure 2: Comparative diagnostic performance of the Serum CRP and ESR measurements in the samples of SAT patients plotted as ROC curve. The dotted lines express the 95% confidence limits. The ROC curves of CRP passed closer to the upper left corner than that of ESR and the AUC of CRP was significantly larger than AUC of ESR (0.774 for CRP (P <0.0038) and 0.751 for ESR (P <0.0110). CRP: C-reactive protein; ESR: Erythrocyte sedimentationrate; SAT:Sub-acute thyroiditis; ROC: Receiver operating characteristics; AUC:Area under the curve

Based on the analysis of ROC curves, we demonstrated that the presence of elevated levels of CRP in patients with SAT has a better diagnostic performance than ESR. The positive predictive value was higher for CRP in comparison to the other marker. We found statistically higher negative predictive value for CRP as compared to ESR. Thus CRP becomes a useful tool in differentiating these two etiological groups convincingly. Although it can be argued that ESR is cheaper and easily available than Serum CRP, the former has poor sensitivity and still poorer specificity.[4] There is a wealth of literature supporting the use of CRP, and to a lesser extent, ESR in the diagnosis and monitoring of treatment of infection in patients.[4,5,26] Furthermore, CRP testing can be been incorporated into newly proposed diagnostic criteria for SAT and proved to be strongly associated with disease. Further studies on larger patient populations are needed to assess the value of CRP in clinical practice, especially for SAT. The study of salivary CRP levels in such two groups with thyrotoxicosis of different etiology may give new insight in this aspect, as indicated by other workers.[12] However we did not have an opportunity to study the same in our patients. A lot of information which is now available suggests that mild rise in serum CRP levels in CAT patients suggest ongoing low grade inflammation.[6,10,11] However, the relationship between the initial titer of this bio marker the long term outcome in SAT patients has not been studied properly, and thus provides impetus for future research in this direction.

Thus the finding of our study proves our hypothesis that raised serum CRP levels underscores the severity of thyroid inflammation and can help differentiate SAT, a grossly inflammatory cause of thyrotoxicosis from Graves' disease which is of non-inflammatory nature.Given that both these conditions may be difficult to differentiate from each other in certain situations, serum CRP can be a simple and predictive test to overcome such dilemma.

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