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C-Reactive Protein as a Marker in the Iraq Patients with Poisoning Thyroid Gland Disease

Abstract- The present study aimed to investigate the possibility of using C-reactive protein (CRP) and the level of CRP in serum sample as a marker for patients with poisoning thyroid gland disease. In addition, the relationship between inflammation and poisoning thyroid gland. About 21 of serum samples were examined from patients with poisoning thyroid gland (aged between 14 and 60 years old). Qualitative test was done to detect the presence of CRP in the patient's serum. The qualitative test showed that 21(100%) patients with poisoning thyroid gland give positive result to CRP. The HPLC analysis done to determine the concentration of CRP in patient's serum. The HPLC analysis showed that the level of concentration for CRP in serum between (6.4-9.49mg/l). According to the results of the present, the significant changes in the levels of CRP for the patients with thyroid disorders observed in current study confirm that inflammation has an important role on pathogenesis of thyroid dysfunctions regardless of their thyroid dysfunction type. As well as the present study shown that, the CRP is a useful marker for patients with poisoning thyroid gland.

Keywords: poisoning thyroid gland, APR, CRP, Inflammation, HPLC

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1. Introduction

Poisoning thyroid gland is most commonly forming of disease in the Iraq country in this time, the rate of occurring in women more than men occur which occur due to the inflammation caused by the autoimmunity and other thyroid diseases are considered unrelated with autoimmune processes. It was previously shown that some APRs levels increase with several thyroid diseases [1]. Acute-phase reactants (APRs) are known with their involvement as pro-inflammatory molecules in various inflammatory diseases. Most of the APRs generally elevate during inflammation C-reactive protein (CRP) is the well-known APRs. Most commonly used APR is C-reactive protein, which is a globulin type protein that use as marker in inflammation, produced by the liver [2,3]. For this reason C-reactive protein consider one of the most important proteins in the medical field and in identifying disease states associated with inflammation. It belonging to pentraxin family of proteins [4]. The Cytogenetic location for CRP gene: 1q23.2 which is the long (q) arm of human chromosome1 at position 23.2, this position contains gene which encode to CRP [5]. It rapidly increases in cases of inflammation and tissue damage, and quickly returns to normal levels as soon as the patient's recovery [6].

The normal level of this protein in serum for perfectly healthy individuals, however, may be (1-5 mg/l) and if it became more than this level this mean there is problem in the health of person [7,8].

The present study aimed to investigate the possibility of using CRP and the level of CRP in serum sample as a marker for poisoning thyroid gland. Moreover, the relationship between inflammation and poisoning thyroid gland.

2. Materials and Methods

I: Blood Sample collection

The sample size of this research paper is 21 human patients with poisoning thyroid gland. For each patient drawn two milliliter of whole blood for patients was obtained under aseptic conditions from each subject by a vein puncture using a disposable syringe. The blood collected in gel tube were separated by centrifugation at 3000 rpm for 10 minutes, for qualitative test and for determine the concentration of CRP in samples by HPLC. The serum samples were subjected to freezing at -20°C [9].

II: Qualitative test

The serum samples containing human CRP were obtained from patients with poisoning thyroid gland, that give positive and negative results for qualitative test by using protocol in the Latex kit

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for C-Reactive protein (Cat. No.: NS 514001/ Salucea Company). The CRP qualitative in human serum according to the following: Shake the CRP

Latex reagent gently and add 100µl of it to the circle on the glass slide and then add 100µ from the patient serum. Then Mix well by using disposable stirrer spreading the mixture over the whole test area and title the slide gently. Agitate for about 2 minutes by hand and observe for the presence or absence of agglutination. If an agglutination of the latex particles suspension will occur within 2 minutes this meaning positive result and if no agglutination will occur this meaning negative result .This kit indicating a CRP level of more than 6 mg/l.

III. Determine the concentration of the C-reactive protein in serum using high-performance liquid chromatography

1) *HPLC*: The HPLC method is low cost and can be obtained at highest sensitivity using to measure very low concentrations in serum [10]. Samples have been analyzed via the system of high performance liquid chromatography (HPLC), model Sigma LC-20AB equipped with binary delivery pump model LC-20AD. The eluted peaks have been observed with UV-Vis detector. The preparation conditions are depicted in Table 1.

2) *Preparation of sample*: After 1min vortex, 100µl from sample (serum) was obtained and injected into HPLC.

3) *Calculation*: The region under a peak is utilized to calculate the concentration of a sample as the following formula [11]:

Concentration of sample (mg/l)=(the area of the sample/area of the standard)×standard conc.

Table 1: Conditions of high performance liquid chromatography

Parameter	Characteristic for CRP identification
Detector	UVspectrophotometer 280nm
Flow rate	1.1 ml/min
Volume injection sample	100µl
Type of column	C18-ODS (10µm,25cm*4.6mm)
Mobile phase	Methanol:deionized water:acetonitrile 80:19:1v/v
Temperature	24°C

4. Results and Discussion

I. Qualitative test

Serum specimens were tested for presence of CRP using latex kit for CRP. The results of qualitative test are shown that 21(100%) patients with poisoning thyroid gland give positive result to CRP this result agreement with studies of [12,13], which significantly that CRP level increase in patients with thyroid disease.

II. Serum CRP concentration

In the present study, it has been developed thorough examination to assess the CRP activity in serum of patients. Figure 1 shows that a complete baseline separation was obtained within C-reactive protein by HPLC.

As it shown in Figures 2 the curves peaks for patients, which were compared with the standard material CRP. Curved of peaks the standard material appeared when CRP analysis at minute (RT=8.58 min) which was its retention time (RT) for the emergence of substance analysis using the above-mentioned conditions. At its time the concentration of CRP patients much more than normal range.

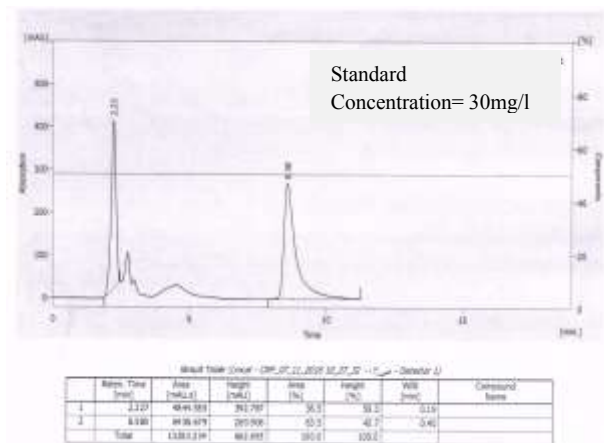


Figure 1: The HPLC Chromatogram signal of Standard CRP level, its retention time (RT) is 8.58 minutes

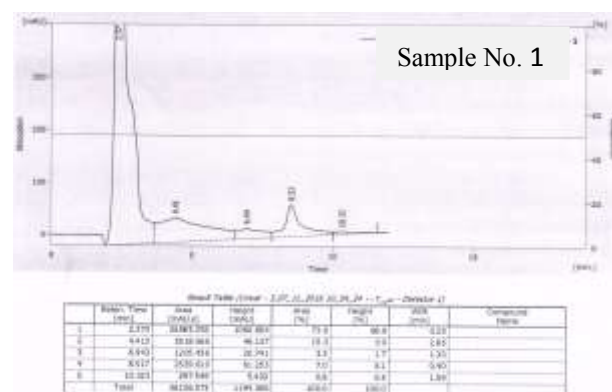


Figure 2: The HPLC Chromatogram signal of CRP Levels in Serum of patient and it's Retention Time (RT= 8.517 minutes).

The concentration of CRP in serum had shown a increase in the patients with poisoning thyroid gland more than normal range (1-5mg/l) [7,8] as shown in Table 2.

Table 2: The concentration of CRP in patients with poisoning thyroid gland

No.	Description	Concentration mg/l
1	Poising thyroid gland	8.99
2	Poising thyroid gland	7.84
3	Poising thyroid gland	7.87
4	Poising thyroid gland	8.79
5	Poising thyroid gland	7.59
6	Poising thyroid gland	6.52
7	Poising thyroid gland	6.67
8	Poising thyroid gland	7.75
9	Poising thyroid gland	8.12
10	Poising thyroid gland	8.69
11	Poising thyroid gland	8.57
12	Poising thyroid gland	9.24
13	Poising thyroid gland	9.3
14	Poising thyroid gland	7.13
15	Poising thyroid gland	9.47
16	Poising thyroid gland	7.84
17	Poising thyroid gland	8.79
18	Poising thyroid gland	7.59
19	Poising thyroid gland	8
20	Poising thyroid gland	8.21
21	Poising thyroid gland	6.4

In present study as shown in Table 2 it's found that CRP has been significantly elevated in patients with poisoning thyroid gland disease were more than normal level (1-5 mg/L). This was in agreement with other studies[7,8] demonstrated that the normal level of CRP in serum sample was (1-5 mg/l) if concentration of CRP increase more than 5mg/l this meaning elevated in CRP level. In addition, this result in agreement with the study [13] demonstrated that CRP was significantly elevated in patients with poisoning thyroid gland. C-reactive protein has several functions that associated with host defense: it promotes agglutination, bacterial capsular swelling, phagocytosis and complement fixation through its calcium-dependent binding to phosphorylcholine (PC) [14]. During inflammation CRP produced as response to IL-6, this explained the relationships between CRP and infection. For this reason CRP was considered as a marker for inflammation [15]. The inflammation consider the common factor that cause thyroid disease this explain the relationship between CRP and poisoning thyroid disease.

5. Conclusions

Significant changes in the levels of CRP for the patients with thyroid disorders observed in current study confirm that inflammation has an important role on pathogenesis of thyroid

dysfunctions regardless of their thyroid dysfunction type. As well as the present study shown that the CRP is a useful marker for patients with poisoning thyroid gland and it can act as marker for predictor and to identify the risk of this disease .

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Author biography



Maryam Dhary Kamel was born in Baghdad, Iraq in March 1992. She received her B.Sc. degrees in 2014 from University of Technology. Her research interest are Bioinformatics, Protein structure prediction, Computational genomics, Molecular

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