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RESEARCH ARTICLE

Investigation of Inflammatory Markers (IL-6 and hs-CRP) in Type1 Diabetes Patients.

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Abstract

The present study was designed to investigate the levels of interleukin-6 (IL-6) and high sensitivity C-reactive protein (hs-CRP) as an inflammatory markers and an association between these markers with the body mass index (BMI) and with the level of glycated hemoglobin (HbA1c) in type1 diabetes patients.

Ninety one blood samples were collected from type1 diabetes patients and healthy individuals in a period between June and the end of November 2012 at the AL-Kindy Specialized Center for Diabetes and Endocrinology and the Central Child Hospital , their ages ranged between (1 - 18 year), they are divided into three groups ,newly diagnosed, chronic and healthy control groups .IL-6 and hs-CRP were done by using ELISA , while HbA1c was done by using spectrophotometer. The results showed that there are significant differences between diabetic patients and non diabetic groups of study ($p \leq 0.05$), highly significant was demonstrated in hs-CRP and IL-6 tests in chronic patients and newly diagnosed patients at mean 9.42 ± 1.28 mg/L and 6.53 ± 2.29 pg/ml respectively .The correlation between hs-CRP and IL-6 with biomass(BMI) ,fasting blood glucose (FBG) and glycated hemoglobin (HbA1c) was non significant ,there was (r= - 0.036 , 0.077,and r = 0.064) for hs-CRP respectively , and (r = 0.022 , 0.90, and r = 0.119) for IL-6 respectively. This study concludes that the circulating levels of inflammatory markers were elevated in patients with T1D and their levels were not affected by glycated hemoglobin A1c and BMI

INTRODUCTION:-

Type 1 diabetes (T1D) is most often diagnosed early in life and is usually the result of an autoimmune attack on the insulin producing β-cells of the pancreas, leading to lack of endogenous insulin secretion. This means that the individuals affected will be in need of life-long insulin treatment for survival (Anna , 2011).T1D is a T cell-mediated autoimmune disease resulting in islet β-cells destruction, hypoinsulinemia, and severely altered glucose homeostasis (Frank and Kevan , 2011 ; Cabrera *et al.* 2012). The resulting progressive destruction of β-cells leads to loss of glucose homeostasis, which results in death if untreated (Mark ,2012). Inflammation is a condition that is common to both obesity and T1D (De Ferranti and Mozaffarin,2008) and systemic inflammation is associated with the development of microvascular and macrovascular complications among persons with type1 diabetes (Lin *et al*.,2008). Measurement of inflammatory markers has two main functions: first one is to detect acute inflammation that might indicate specific diseases, the second is to detect a marker of treatment response (Jessica *et al*., 2012). Several acute-phase inflammatory markers have been reported to be increased in type 1 diabetes. IL-6 is a proinflammatory cytokine that is elevated in both type 1 and type 2 diabetes (Wedrychowicz *et al*.,2004), and may be increased by hyperglycemia. High-sensitivity C-reactive protein (hsCRP) is an acute-phase protein that is associated with systemic inflammation (Janet *et al*., 2010). Cytokines are regulatory proteins produced and secreted

by lymphocytes and monocytes (Karlsson *et al*., 2004)**.**IL-6 is pleiotropic cytokine with a key impact on both immune regulation and none outside the immune system whether inflammation plays a role in the pathogenesis of early diabetic patients(Nikhil and Ravinder,2008). The HbA1c test is currently one of the best ways to check diabetes is under control (Osama *et al.*,2013).

The aim of this study is to investigate serum concentrations of interleukin-6 (IL-6),and high sensitivity-C reactive protein (hs-CRP) in individuals with T1D , and to determine their correlation with HbA1c and body mass index (BMI) in T1D patients.

Material and Methods

Subjects and Methods:

Patient Group

This group included 66 subjects for hs-CRP and for IL-6 test those were suffering from T1D after fasting blood glucose and HbA1c tests were done in a period between June and the end of November 2012 . They were selected from the AL-Kindy Specialized Center for Diabetes and Endocrinology and the Central Child Hospital. Their ages ranged from 1-18 years, with mean age of(7.7 years ± 4.2)for short duration ≤ 3 months (newly diagnosed) group and mean age of (11.75 ± 4.19 years) for long duration >3 months (chronic) group.

Healthy control group

Twenty five healthy individuals with no family history of diabetes were considered as a control group. They were free from any systemic disease. Their ages ranged from 5-16 years with a mean age of (11.5 ± 6.3 years).

Anthropometric measures

Height was measured in centimeters using standiometer. Weight was measured in kilograms using an electronic scale According to the Quettletś equation (Frier *et al.*, 1999). BMI was calculated using measured weight (kilograms) divided by measured height (meters) squared (kg/m^2) .

Laboratory analysis

Venous blood(3 ml) was withdrawn from each subject of the three groups . Some of blood put in EDTA tube used for quantitative colorimetric determination of glycohemoglobin A1c in whole blood (HbA1c) analysis (using a kit supplied by STANBIO/Boerne, Texas cat. No 0355), and measurement absorbance at 415 nm using by spectrophotometer (CECIL 2031/France). The remaining blood was left to clot for 15 minutes, centrifuged and serum was separated some was used immediately for a glucose oxidase method of fasting blood glucose (FBG) analysis (supplied by GLU-PAP/United Kingdom),and measurement absorbance at 500nm by using spectrophotometer.

The remaining serum was kept frozen at –20°C for the subsequent assay of hs-CRP using by Enzyme Immunoassays for the Quantitative High Sensitive Determination of C-Reactive Protein in Human serum (using a kit supplied by Demeditec Diagnostics cat. No.DE740011) ELISA Kit , and of IL-6 assay was done by using Enzyme Immunoassays for the Quantitative determination of human interleukin - 6 concentrations in serum (using a kit supplied by CUSABIO cat. No.CSB-E4638h) ELISA Kit .

Statistical analysis

Statistical Analysis System – SAS (2010) program was used to detect the effect of difference factors in study parameters. The linear relationship between variables was assessed by Pearson's correlation coefficient (r). For all tests, P values less than 0.05 were considered statistically significant .

Results

Clinical and physical characteristics of patients with T1D and control subjects were demonstrated in table (1). There was a significant difference in BMI in diabetic patients as compared with control group. However, comparison of serum glucose levels and glycosylated hemoglobin (HbA1c) between patients with diabetes mellitus and healthy subjects were shown in table (1). The results showed that serum glucose and HbA1c levels were significantly higher in diabetic patients than healthy subjects ($P \leq 0.05$).

As can be seen from the table (2) the mean values of serum inflammatory cytokines [hs-CRP and IL-6] levels were extremely significant in diabetic patients compared to control group ($P<0.001$). The relationships between serum inflammatory cytokines and all clinical parameters with patients T1D and healthy subjects were non significant in (Table 3,4) .Non significant correlation between inflammatory markers (IL-6 and hs-CRP) were also found. Figure(1) showed that HbA1c was increased in the newly diagnosed group than other study groups .The concentration of IL-6 was elevated in the newly diagnosed group (figure 2).Whereas highest level of hs-CRP was found in the diabetic patients with long duration group than other groups this determined in (figure 3).

Table 1:-Distribution of Biomass, FBG and HbA1c in study groups of T1D

GROUPS	N	BIOMASS Kg/m^2 Mean ± SE	FBG mg/dl Mean ± SE	HbA1c % Mean ± SE
Healthy control	25	20.58 ± 0.74^a	92.64 ± 1.07^c	5.0 ± 0.11^c
Newly diagnosed patients	33	18.07 ± 0.69^c	290.45 ± 25.86^a	10.3 ± 0.36^a
Chronic patients	33	19.91 ± 0.81^b	204.48 ± 15.23^b	7.43 ± 0.29^b

FBG : Fasting Blood Glucose,
HbA1c: Glygated Hemoglobin A1c. index BIOMASS: Body mass

Table 2:- Determination of inflammatory markers(IL-6 and hs-CRP) in serum of study groups of T1D.

GROUPS	N	CONCENTRATION hs-CRP(mg/L) Mean ± SE	CONCENTRATION IL-6(pg/ml) Mean ± SE
Healthy control	25	1.59 ± 0.21^c	0.48 ± 2.61^c
Newly diagnosed patients	34	7.42 ± 1.18^b	6.03 ± 2.29^a
Chronic patients	29	9.42 ± 1.28^a	2.58 ± 1.30^b

Table (3):- The Correlation between IL-6 with other Parameters in T1D patients.

Marker / Parameters	IL-6	N	p-value
BMI	0.022	91	NS
FBG	0.90	91	NS
HbA1c	0.119	91	NS
Hs-CRP	-0.159	76	NS

NS= Non significant.

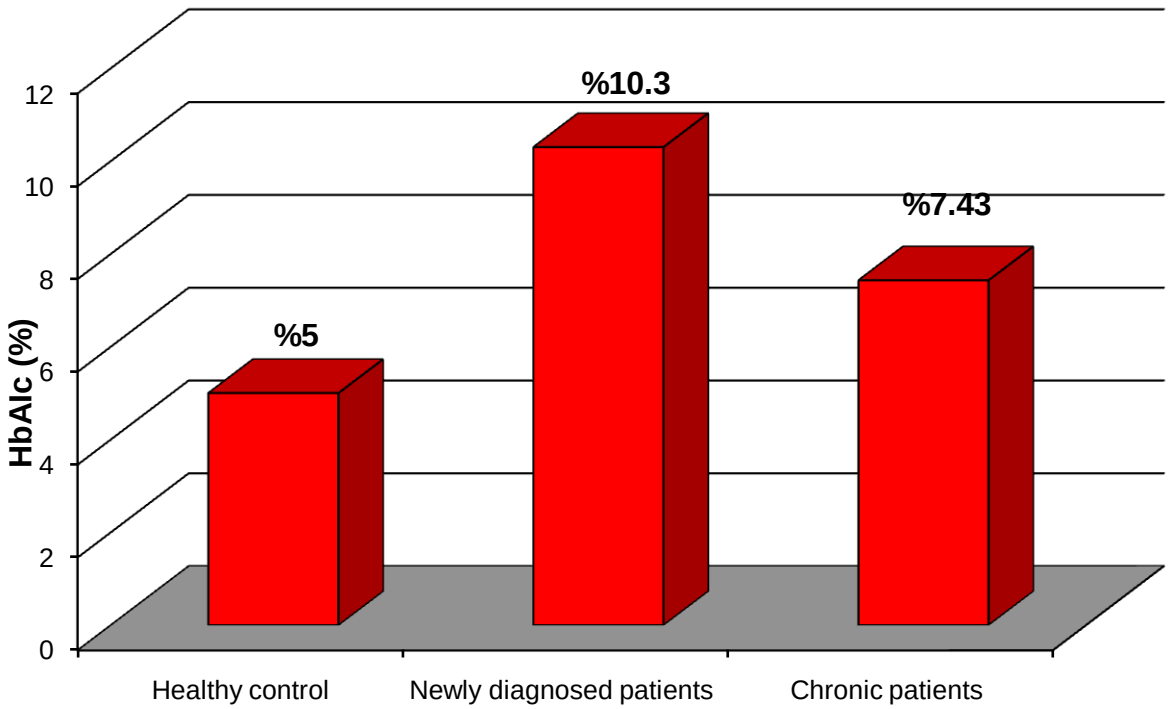

Figure 1. The percentage of HbA1c (%) in study groups of T1D patients compard to control.

Table (4):- The Correlation between hs-CRP with other Parameters in T1D patients.

Marker / Parameters	Hs-CRP	N	p-value
BMI	-0.036	91	NS
FBG	0.077	91	NS
HbA1c	0.064	91	NS
IL-6	-0.159	88	NS

NS= Non significant

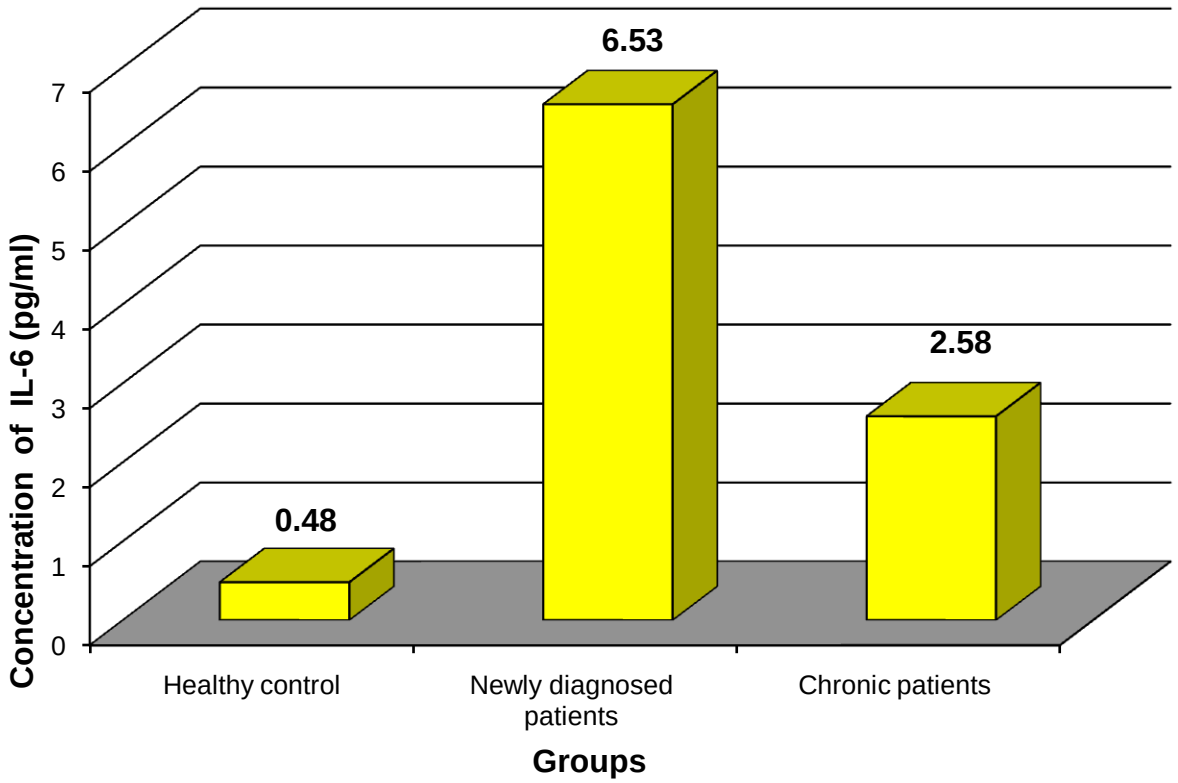

Figure 2. Concentration of inflammatory marker IL-6 in serum of T1D patients compared to control by ELISA

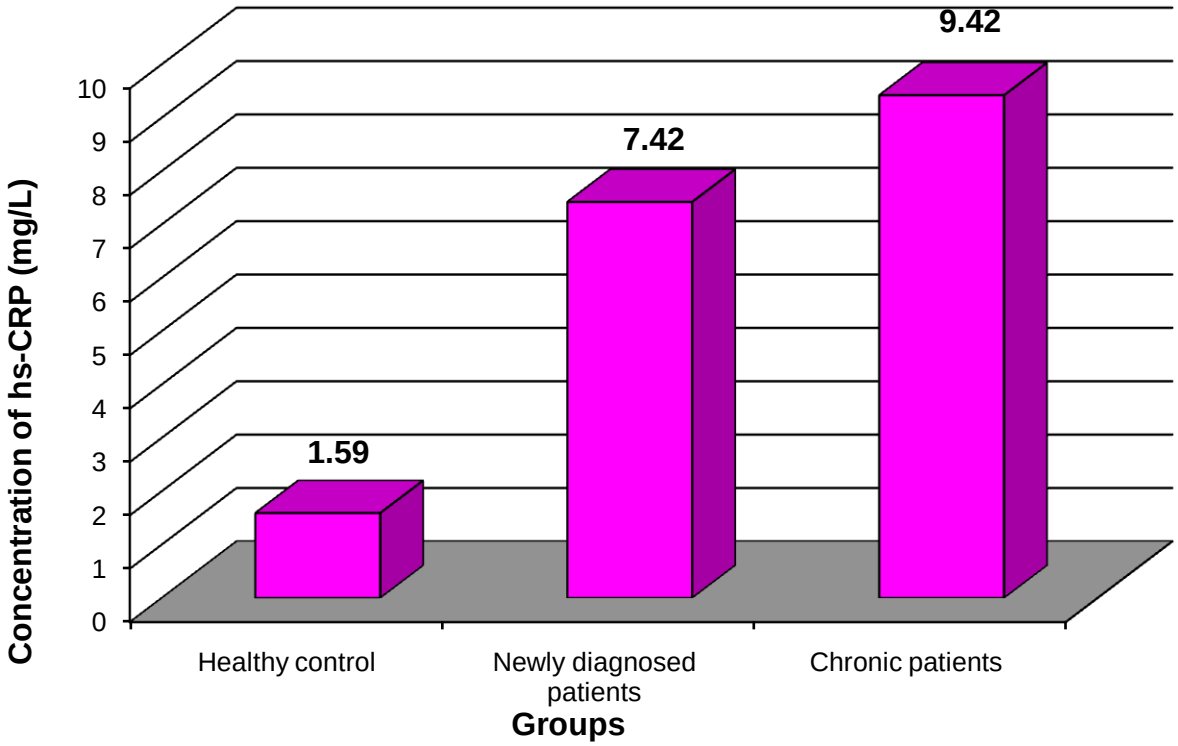

Figure 3. Concentration of inflammatory marker hs-CRP in serum of T1D patients compared to control by ELISA

Discussion:

The proinflammatory cytokines IL-6 play an important role in the pathogenesis of insulin- development diabetes mellitus (*Alexandraki et al.*,2008) .

Data of the present study revealed that serum cytokines IL-6 and hs-CRP levels were significantly higher in patients of type1 diabetes compared to healthy control. These results are in agreement with those of (Wasmuth *et al.,* 2004, Dandona *et al.*, 2004, Glowinska & Urban, 2003 and Azza *et al*.,2010 ; Janet *et al*.,2010) who reported that the inflammatory activity is increased in individuals with type-1 diabetes because inflammation involves the release and increased activity of various immune system cells, T-cells and macrophages . The present study observed high significant differences in Biomass , FBG , and HbA1c at (p≤ o.o5) between study groups. Higher levels of FBG and HbA1c in newly diagnosed patients were found compared with healthy controls because some cases are smaller in age may be related to rapid changes in lifestyle, and the symptoms might be lately diagnosed . These results are in agreement with those of (William,2009 ; Osama *et al*., 2013) ,while good control appeared in chronic patients because correct diabetes care as well as control of HbA1c and treatment with insulin in these cases more than the newly diagnosed group , thus the HbA1c is widely used as an index of mean glycemic in diabetes , as measure of risk for the development of diabetes complications , and as a measure of the quality of diabetes care these in agreement with William and Robert,(2012). Whereas Shalitin ,and Peter(2011) observed the HbA1c levels were lower in the treatment groups($p<0.05$). However, Elevated in IL-6 and hs-CRP concentrations among patients with T1D , because acute hyperglycemia and worse glycemic control have been associated with increased inflammation in an individual with T1D , this study agree with Aribi *et al*.(2007) .Also the high level of glucose may be activate p38 MAPK (Mitogen Activated Protein kinase) which regulate the production of inflammatory cytokines such as hs-crp and IL-6 this agrees with Igarashi *et al.,*(1999). On the same line Karlsson *et al*.,(2004) ; Sridevi *et al. (*2005); reported that under high glucose, monocytes secrete increased amounts of IL-6 .Results in the present study demonstrated there was no significant correlation has been found between IL-6 and HbA1c (r= 0.119) ,this result

was agree with Rosa *et al*.,(2008) and non agreement with Katherine *et al*.,(2002); who found significant correlation of IL-6 levels with HbA1c may be even patients with good glycemic control elevated level of IL-6 . Also there was no significant correlation between inflammatory markers and Biomass, FBG this data agrees with study of Snell *et al*., (2010) .The good glycemic control decreases the level of inflammatory markers which probably plays a role in elevated inflammation and the risk of cardiovascular development in T1D patients, as well as environmental factors play a role in diabetes outcomes including glucose control .The highest levels of hs-CRP was found in chronic patients may be related with complications in persons with T1D including the development of retinopathy, nephropathy and premature atherosclerosis this result agree with previous studies of Soliman, *et al*.,(2002); Mangee, *et al*.,(2004); Zorena, *et al*.,(2007); Lin ,*et al*.,(2008). Previous study was found that levels of IL-6 ,hs-CRP and HbA1c significantly decreased in all groups after 6 months of treatment as being found by Nikhil and Ravinder (2008), because insulin is one of the islet autoantigen responsible for activation of T-lymphocyte functions, inflammatory cytokines production and development of T1D. Another study obtained by *Chatzi et al. (*2010) found that the duration of diabetes was associated with general scores of inflammatory markers. Unlike previous studies, our data indicated that the elevation of cytokine markers like IL-6 and hs-CRP were comparable in diabetic patients with a shorter duration of disease more than in diabetic patients with a longer duration of diabetes .

Conclusion :-

In conclusion, circulating levels of high sensitivity C-reactive protein and interleukin-6 are significantly increased in patients with T1D compared to healthy subjects and their levels are not affected by glucose level. This is highly suggestive of the availability of these non invasive indices to help in further examining Type 1DM pathophysiology and monitoring pharmacological interventions to interfere with disease development and progression.

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