

RELATIONSHIP BETWEEN HBA1C LEVELS AND INFLAMMATORY BIOMARKERS (C - REACTIVE PROTEIN, IL6 AND TNF-ALPHA) AMONG TYPE 2 DIABETES MELLITUS-KHARTOUM- SUDAN

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Abstract

Introduction: World most prevalence disease, is diabetes mellitus, with countless causes, it on increasing, genetic, lifestyle, infections could all be contributors as causative agents especially type 2 diabetes mellitus, increase inflammatory biomarkers plays extremely critical and vital role in progression of type 2 diabetes mellitus. So this study aimed to assess glycated hemoglobin levels and inflammatory biomarkers among T2DM and find such correlation and compare data with healthy control.

Method: Cross-sectional case control study among 100 case group T2DM and 100 healthy subjects as control group, HbA1c and inflammatory biomarkers, C-reactive protein, IL6 and TNF-alpha were measured for study groups. Enzyme linked immunoassay (ELISA) used for measurement of inflammatory biomarkers TNF- α , CRP, IL6 and Cystatin C. while HbA1c assessed by ichroma device.

Result: HbA1c, C-reactive protein, IL6 and TNF-alpha were increased among T2DM patients than healthy subjects, giving increased significant difference for each, as p value <0.05. Pearson's correlation of HbA1c with each of measured parameters showed that HbA1c has positive correlation with inflammatory biomarkers TNF- α . (R= 0.812** P=0.00), CRP(R= 0.527** P=0.00), IL6, (R= 0.778** P=0.00) and Cystatin C (R= 0.884** P=0.00) respectively.

Conclusion: higher levels of inflammatory biomarkers associate with increase with glycated hemoglobin, Improvement of glycemic control reduce risk markers for development of diabetic complications.

Key words: HbA1c Levels and inflammatory biomarkers, tumor necrosis factor, interleukin 6.

Introduction:

Diabetes mellitus is a group of metabolic diseases with chronic hyperglycemia resulting due to defects in insulin secretion, insulin action,

or both. Metabolic abnormalities in carbohydrates, lipids, and proteins result from the importance of insulin as an anabolic hormone. Low levels of insulin to achieve adequate response and/or insulin resistance of

target tissues, mainly skeletal muscles, adipose tissue, and to a lesser extent, liver, at the level of insulin receptors, signal transduction system, and/or effector enzymes or genes are responsible for these metabolic abnormalities. The severity of symptoms is due to the type and duration of diabetes. Some of the diabetes patients are asymptomatic especially those with type 2 diabetes during the early years of the disease (1-2). Due to its increasing incidence (3), diabetes mellitus is currently the leading cause of chronic kidney disease (4); approximately 40% of patients with diabetes develop diabetic kidney disease (5). Chronic kidney disease accounts for nearly half of all incident cases of end-stage renal disease in the United States and 5-year survival for patients with end-stage renal disease is <40% (6). It has been proposed that inflammatory cytokines secreted by adipose tissue exert an endocrine effect conferring insulin resistance in liver, skeletal muscle, and vascular endothelial tissue, ultimately leading to the clinical expression of both type 2 diabetes and cardiovascular disease (CVD) (7-8). In particular, elevated production of adipocyte cytokines, such as tumor necrosis factor (TNF)- α and interleukin (IL)-6, leads to an acute-phase response with increased hepatic production of C-reactive protein (CRP), a sensitive marker of low-grade systemic inflammation (9-10). TNF- α , IL-6, and CRP not only directly promote insulin resistance, but also stimulate endothelial production of adhesion molecules such as E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular adhesion molecule-1 (VCAM-1), critical mediators of endothelial dysfunction in capillary and arteriolar endothelium (11). Potential implication of the many studies suggesting a relation between inflammation and diabetes is that inflammatory markers may be used to refine diabetes risk prediction and thus better target individuals for lifestyle interventions (12-13). Two previous meta-analyses evaluating the association of CRP and diabetes risk have yielded contradictory results. One previous meta-analysis (14) suggested that a positive association exists between CRP and diabetes risk.

In contrast, another meta-analysis (15) concluded that CRP may not be an independent risk factor for the development of diabetes.

Material and method

This cross-sectional case control study targeted T2DM patients to assess blood glucose, glycated hemoglobin (HbA1c) and inflammatory biomarkers CRP, IL6 and TNF- α . 100 patients with T2DM were recruited, their age varied between 33-55 years. Another 100 individuals also enrolled, they were healthy set as control group. Glucose level was measured spectrophotometrically by means of BTS350 device, Biosystem trade mark, accompanied with reagent for glucose at wave length 520 nm, HbA1c was measured with ichroma device and suitable kit, it based on immunodetection principle. While inflammatory biomarkers CRP, IL6 and TNF- α were assessed by enzyme linked immunoassay (ELSA). Laboratory work conducted at modern medical laboratory-Khartoum.

Exclusion Criteria

Patients with smoking habit, acute inflammations, acute infections, chronic liver diseases and chronic diseases were excluded from this study.

Ethical consideration: This study was approved with the ethical committee of Alneelain university- faculty of graduate and medical laboratory science. Every diabetic center and personnel involved did give consent to be recruited for this study.

Statistical analysis: Data obtained was analyzed using statistical package of social science (SPSS) version 22. Fasting blood glucose, HbA1c and Inflammatory biomarkers were used for statistical analysis. All the data were expressed as mean \pm SD (standard deviation), the statistical significance of differences between the values was assessed by ANOVA or Mann-Whitney U test (as appropriate). Logistic and Multiple regressions were performed among on the parameters. A two-tailed p value of <0.05 was considered as statistically significant.

Result

In this cross-sectional case control study; 100 T2DM and 100 healthy individuals (control group) were enrolled to assess biomarkers for infection, beside glucose and glysated hemoglobin. The T2DM patients were sorted according to age to more than 50 years 36 (36%)

and less 50 years were 74 (64%). for each group they were 50% males and 50% females. Comparing measured parameters for case group and control group revealed increased levels of all parameters and brought significant difference for each one, as p value <0.05 as in table 1.

Table 1: mean comparison of study parameters in case versus control

Parameters	Case (Mean±SD)	Control (Mean±SD)	P-value
FBG	180.30±35.91	96.36±9.49	<0.01
HBA1c	7.74±1.12	5.28±0.37	<0.01
s-CRP	6.05±2.47	3.18±1.67	<0.01
IL-6	13.96±3.72	11.72±2.56	0.001
TNF-α	13.50±3.11	9.02±1.92	<0.01

Significant difference p value <0.05

Considering gender, parameters of case group compared between male and females, no significant difference was obtained all p values were >0.05 as in table 2

Table 2: mean comparison of study parameters across gender

Parameters	Male (Mean±SD)	Female (Mean±SD)	P-value
FBG	183.92±38.15	176.68±33.90	0.482
HBA1c	7.47±1.00	8.00±1.18	0.090
s-CRP	6.52±2.47	5.59±2.43	0.189
IL-6	13.74±4.24	14.19±3.20	0.675
TNF-α	13.06±3.42	13.93±2.75	0.328

Significant difference p value <0.05

The conduct of Pearson’s correlation between measured parameters and age of case group members, showed that positive correlation (i. e both increased or both decreased) between age with HbA1c, and negative correlation with glucose, IL6, CRP and TNF-α as in table 3

Table 3: correlation between age and study parameters

Parameters	R-value	P-value
FBG	-0.059	0.683
HBA1c	0.008	0.955
CRP	-0.068	0.637
IL-6	-0.085	0.556
TNF-α	0.037	0.796

Significant difference R value -1 and +1

Also Pearson’s correlation of HbA1c with each of measured parameters, showed that HbA1c has positive correlation with TNF- α , cystatin c, CRP, IL6, as in figures (1, 2,3 and 4) respectively. Correlation with HbA1c gave same ones.

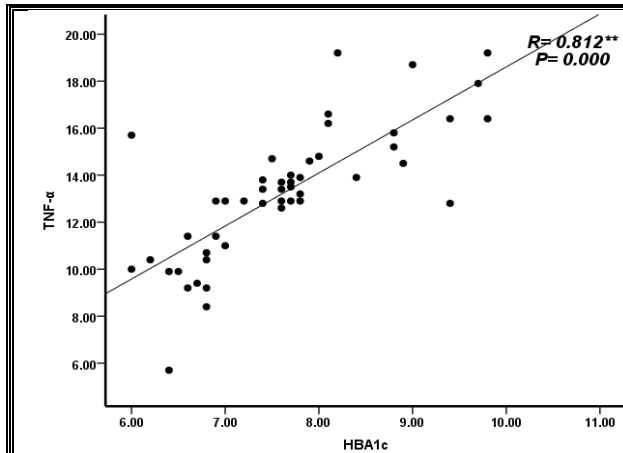


figure 1: HbA1c& TNF- α (+ve correlation)

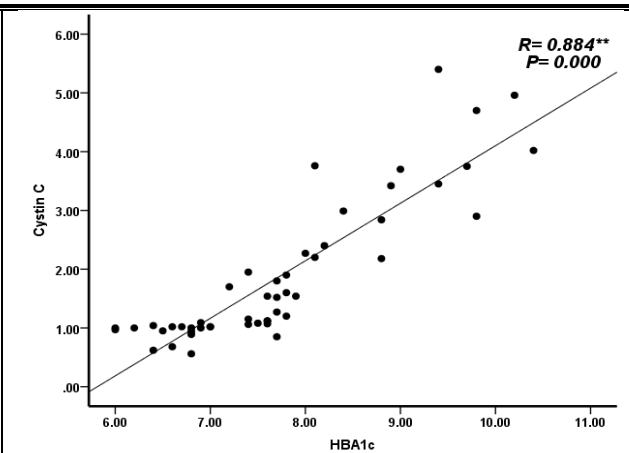


figure 2: HbA1c& cystatin c (+ve correlation)

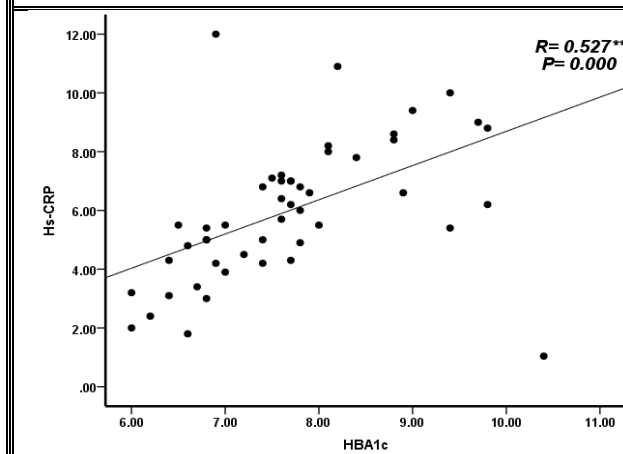


figure 3: HbA1c& CRP (+ve correlation)

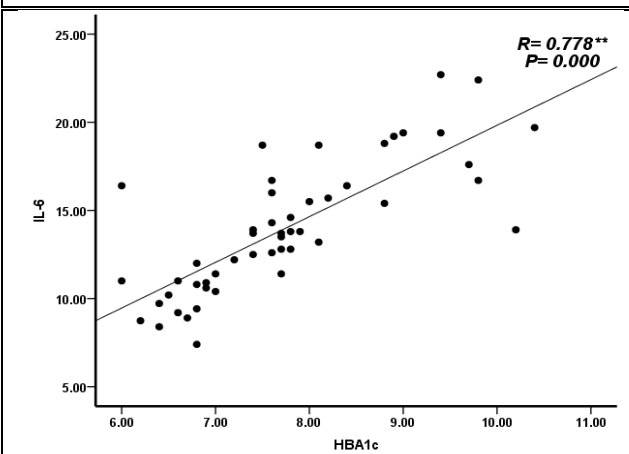


figure 4: HbA1c& IL6 (+ve correlation)

Discussion

The rapid increase in the prevalence of type 2 diabetes has become a serious public health problem (16). T2DM can be accompanied by long-term microvascular and macrovascular complications, which lead to both morbidity and mortality (17). One-third of individuals with type 2 diabetes are undiagnosed. However, accumulating evidence shows that inflammation may play a crucial intermediary role in the pathogenesis of type 2 diabetes, thus relating diabetes to a number of commonly coexisting conditions thought to originate via inflammatory mechanisms (18). In this regard, more recent data suggest that interleukin-6 (IL-6) and C-reactive protein (CRP) are associated with type 2

diabetes (19-20). IL-6, a pleiotropic proinflammatory cytokine, is produced by a variety of cells, including activated leukocytes, endothelial cells, and adipocytes (21). CRP is an acute-phase plasma protein synthesized by the liver and has been shown to be a sensitive, systemic biomarker of inflammation (18). The stability of this protein during long-term frozen blood storage and the availability of inexpensive, precise, and standardized assays have assisted studies of CRP (22). TNF α is a powerful pro-inflammatory agent that regulates many facets of macrophage function. It is rapidly released after trauma, infection and has been shown to be one of the most abundant early mediators in inflamed tissue (23) A high level of TNF- α is

believed to induce insulin resistance and is considered to contribute to the development of diabetes (24). In 2013, reported that, increased levels of TNF- α (13.5 pg/ml) were associated with severity of diabetic complications (25). While the concentration of serum IL6 normally fluctuates according to physiological conditions and rapidly returns to basal levels, it becomes chronically elevated with T2DM (26).

In this study, already diagnosed individuals were involved to assess biomarkers of inflammation, CRP, IL6 and TNF- α , beside glucose levels and HbA1c, compared with data of healthy subjects, all measured parameters were elevated, giving clue about persistent infection or inflammation among T2DM, inflammatory biomarkers were positively correlated with levels of glucose and HbA1c. An agreement found with study, suggested the elevated levels of inflammatory markers, including C-reactive protein (CRP), tumor necrosis factor alpha (TNF- α), and interleukin 6 (IL6) are supposed to be associated with type 2 diabetes mellitus (T2DM) and aimed to determine the difference of these inflammatory markers as well as GL in individuals with versus those without T2DM in rural Thais. A total of 296 participants aged 35–66 living Thailand, were recruited. Blood was collected to evaluate blood glucose levels, and inflammatory markers. Elevated CRP and IL6 levels were associated with increased risk of developing T2DM. There was a trend towards increased risk of T2DM with elevated TNF- α levels and conclude that, CRP, IL6, and TNF- α associated with T2DM (27). Also other study aimed to investigate the association between elevated tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) and high sensitivity-C-reactive protein (hs-CRP) with type 2 diabetes mellitus (T2DM) in abdominal obesity women subjects. A total of 428 abdominal obesity subjects (age 48.4 ± 10.2 years), and 107 non- abdominal obesity women subjects (age 48.8 ± 11.8 years) were enrolled for the all biochemistry testing, inflammatory cytokines, plasma glucose and inflammatory cytokines. Elevation of TNF- α , IL-6, hs-CRP and insulin resistance were significantly associated

with T2DM in abdominal obesity subjects, after adjusting with insulin resistance, suggesting that inflammatory cytokines were positively associated with T2DM and may have a causal relation with an increased oxidative stress and insulin resistance in these abdominal obesity women subjects (28). A partial agreement with Ghanaian study determined the variation levels of IL-6 and TNF- α in T2DM patients. A nested case–control design using participants aged 25–70 years consisting of 77 T2DM \pm hypertension patients and 112 controls were selected from a larger study on Research on Obesity and Diabetes among African Migrants (RODAM). Anthropometric measurements, blood pressure and body fat percentage were measured. Fasting blood samples were analyzed for glucose, IL-6 and TNF- α levels. The median level of IL-6 was significantly higher ($p < 0.0001$) among rural dwellers compared to urban dwellers. Inversely, urban dwellers had significantly higher ($p = 0.0424$) median level of TNF- α compared to rural cases. No significant differences were observed in IL-6 ($p = 0.3571$) and TNF- α ($p = 0.2581$) among T2DM patients compared with T2DM \pm hypertension patients, the study suggested that there was no association of body fat percentage and body mass index with IL-6 and TNF- α levels. Co-morbidity of hypertension with T2DM had no effect on IL-6 and TNF- α level (29). In previous studies, the relationship between inflammatory markers and glycemic control is still not fully understood. there's no inflammatory markers correlated with HbA1c levels. These results are consistent with the findings of previous cross-sectional studies which have found an inconsistent association between inflammation and blood glucose levels (30-33).

Conclusion

Present study concluded that the levels of inflammatory markers such as CRP, IL6 and TNF- α were considerably elevated in T2DM patients than to normal control and positively correlated between of glycated hemoglobin and inflammatory biomarkers CRP, IL6 and TNF- α in T2DM patients. The study provides inflammation

is considered to play an important role in progression of T2DM, the first clinical evidence that inflammatory biomarkers CRP, IL6 and TNF- α - and Fas-mediated pathways are strongly associated with complications of T2DM patients. Improvement of glycemic control reduces the complications of T2DM patients.

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