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IMPACT OF SOME INFLAMMATORY MARKERS (CRP & IL8) ON ANEMIA IN NEGATIVE MICROALBUMINURIC DIABETICS

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ABSTRACT

Diabetes represent's the most common cause of renal anemia. Among the reported risk factors for the increased prevalence of anemia in both types of diabetes is the inflammation. C- reactive protein (CRP) was formerly regarded as solely a biomarker for inflammation, is currently regarded as a prominent partaker in endothelial dysfunction, as well as, in atherosclerosis. Besides the previously reported that, chronic elevation of glucose levels causes induction of interleukin -8 (IL-8). These observations were analyzed and possible existence of significant correlations between anemia modulator; serum hepcidin levels and inflammatory markers (high sensitive C- reactive protein and Interleukin-8) in anemic type1and type 2 diabetics with negative microalbuminuria. Eighty-six subjects were included, 20 of them were of type1 diabetes mellitus, 34 of type2, in addition to 32 of apparently healthy subjects as the control group. All of the diabetics were selected to have anemia (Hb<13g/dl) , without overt kidney disease (-ve microalbuminuria test). The result showed that Serum hepcidin levels exhibited significant elevations in both types of diabetes (>43% in type1 and ~35% in type 2). Serum hs-CRP levels were significantly lowered in type 1, wheareas its level was highly elevated in type 2diabetics.In contrary to IL-8 values were significantly elevated in type 1 specifically. Pearson's correlations studies exhibited significant correlations between CRP and renal function indicators; creatinine clearance (CCL) and serum urea in type 2,while IL-8 seems to be more related to glycemic index :Hb_{A1C} and to CCL , in type1 diabetes.

KEY WORDS: *Diabetes mellitus*, Anaemia, CRP , IL-8, Hepcidin , Microalbuminuria.

INTRODUCTION

Diabetes is the most common cause of chronic kidney disease (CKD) , present in nearly two thirds of all patients with renal impairment . Meanwhile, anaemia represent's a common complication of CKD, affecting over half of all patients [1]. Consequently, diabetes could be also the most common cause of renal anaemia [2]. It was found that anaemia develops earlier in patients with diabetes than in patients with renal impairment from other causes [3]. Like many of the pathophysiological changes of diabetic nephropathy, such as albuminuria, anaemia may be apparent even before a demonstrable decline in renal function can be detected [4]. Generally, a normochromic , normocytic anaemia has been observed in diabetic patients without overt renal disease [5]. Thus anaemia has the potential to adversely affect the health of patients with diabetes in a variety of ways [6] .Indeed, much of the impaired quality of life and morbidity previously suffered by patients with renal failure may have been as a consequence of renal anaemia, i.e. for patients with diabetes, anemia constitutes an unwelcomed additional burden [7]. Among the reported risk factors for the increased prevalence of cardiovascular diseases (CVD) in diabetes are hypertension, smoking, obesity, malnutrition, hypoalbuminemia, hyperhomocysteinemia, inflammation and oxidative stress [8] . Because microvascular disease, one of the most prevalent is atherosclerosis, is currently regarded as a dynamic & progressive disease arising from the combination of endothelial dysfunction & inflammation [9]. Where C- reactive protein (CRP) was formerly regarded as solely a biomarker for inflammation ,

is currently regarded as a prominent partaker in endothelial dysfunction as well as in atherosclerosis [10]. However, CRP is no further considered as only a biomarkers , but also as a proatherosclerotic molecule , which mediate it's effects upon the endothelium through the production of interleukin -8 (IL8) [11] . IL8 is a member of chemokines , promotes monocyte - endothelial cell adhesion & arrest , it is abundant in atherosclerosis plaques [12]. The fact that Interleukin-8 secretion is increased by oxidative stress, which thereby causes the recruitment of inflammatory cells, induces a further increase in oxidant stress mediators, making it a key parameter in localized inflammation [13] . On the other hand, the pathogenesis of anemia of inflammation, as that of diabetes, and the regulation of iron absorption and distribution rank among the major unsolved problems . In the last years, a rapid progress has been made on these problems by elucidating the central role of hepcidin, an iron-regulatory hormone and a mediator of innate immunity [14]. Studies of the molecular mechanisms of hepcidin activity could transform our understanding of the regulation of iron transport and may lead to new approaches in treatments of hemochromatosis and anemia of inflammation [15].

Inspired by these observations, in the present study we analyzed the possible existence of significant correlations between anemia modulator; serum hepcidin levels and inflammatory markers(high sensitive C- reactive protein and Interleukin-8) in anemic type1and type 2 diabetics

without overt diabetic nephropathy (negative microalbuminuri).

SUBJECTS & METHODS

A total number of 86 subjects were included in the study, twenty of them were of type 1 diabetes mellitus, thirty – four with type 2 diabetes, in addition to 32 of apparently healthy subjects as a control (table-1) . All of the diabetics were selected to have anemia (Hb < 13 g/dl for males and <12g/dl for females [16]), those were without overt kidney disease (Glomeruler filteration rate- GFR > 60 ml/min and negative microalbuminuria test [17]) , from those patients attending The National Center for Diabetes /Al Yarmook Teaching Hospital – Baghdad/ Iraq- 2010 , , during their periodic visits . Patients were kept on their regular antidiabetic therapy under supervision of specialist physicians. Fasting blood specimens were obtained after an overnight fasting , EDTA tubes were used for .

hematological studies(Hb , MCV, RBC count) utilizing Cell-DYN Ruby® Hematology analyzer (Abbott Diagnostics, USA), and glycated Hb (HbA1C) was estimated based on HPLC by the variant® (Bio-Rad USA) . Serum was separated for measurement of : glucose [18] ,urea [19], creatinine [20], hepcidin was estimated by ELISA assay[21] as well as , hs-CRP [22] and IL8 [23]. First morning urine specimens were obtained to test for microalbuminuria using semi-quantitative urinary strips purchaced by Roche Diagnostics®, based on immunological detection of human albumin by means of soluble monoclonal antibodies against human albumin – gold conjugate on strips to be immersed into the first morning voided urine [24]. The study was approved by The Local Research Ethics Committee and all subjects were given a written informed consent to participate in this study. Statistical analysis was performed by SPSS Version 17

TABLE 1: Descriptive Characteristics of Subjects Included in The Study

Character	Control	Type 1 DM	Type 2 DM
Number	32	20	34
Age (yrs)	36.161±2.235	21.736±1.086	48.000±1.078
Sex(male/female)	14/18	7/13	18/16
BMI (kg/m^2)	18.65±3.73	19.77±4.65	24.43±5.21 *
Fasting Serum Glucose (mg/dl)	92.441±2.012	241.850±20.863**	174.592±9.357**
Fasting Serum Insulin (μIU/l)	8.78±0.32	12.36±1.49**	18.14±1.58**
GlycatedHeamoglubin (%)	5.421±0.0966	9.088±0.287**	11.771±0.424**
Serum Urea (gm/dl)	33.812±1.334	35.681±4.053	34.442±1.604
CCL (ml/min)	106.366±3.100	95.899±4.850	85.779±3.624**
Hemoglubin (gm/dl)	14.809±0.233	11.820±0.409**	12.194±0.209**
MCV (fl)	86.292±0.664	79.156±1.665**	84.561±0.789
RBC count (10^6 /ml)	5.273±0.106	4.797±0.195*	4.994±0.097

Data are presented as Mean ± SEM , * = significantly different from control(p<0.05), **= significantly different from control(p<0.001) .yrs=years , BMI=Body Mass Index , CCL= Creatinine Clearance, MCV=Mean Corpascular Volume , fl=femtoliter , RBC=Red Blood Cells , DM= Diabetes mellitus .

RESULTS

As summarized in table -1, the participated diabetic patients (both type 1&type2) were presented with elevated fasting serum glucose values as compared to controls, which is parallel to the elevated Hb$_{AIC}$ levels.

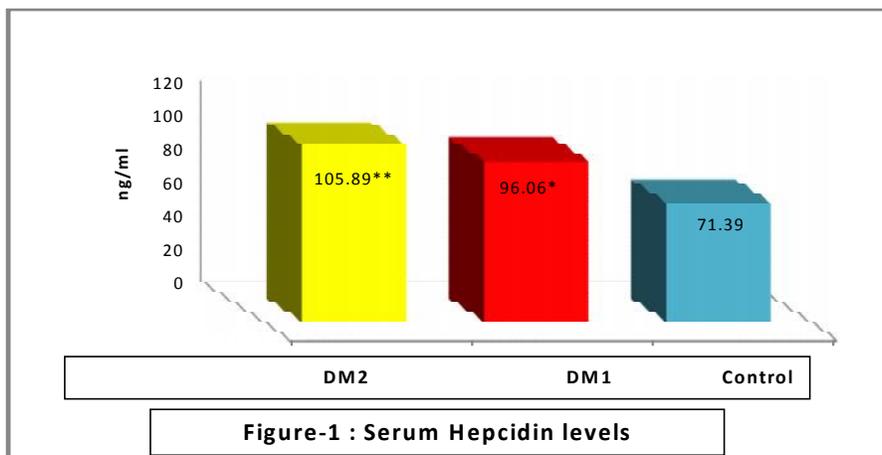
Figure-1 : Serum Hepcidin levels

Data are presented as mean values , DM2 = Type 2 Diabetes Mellitus , DM1 =Type 1 Diabetes Mellitus. *=Significantly different from control (p<0.05) ,**= Significantly different from control (p<0.001).

Although serum urea levels were not significantly altered, creatinine clearance (CCL) values were significantly lowered in type 2 diabetics (by > 19%) despite the negative microalbuminurea results . The anemia of the selected of type 1diabetes patients was microcytic type (MCV less than controls). Serum hepcidin levels were significantly elevated in diabetics (by>43% in type 1and about 35% in type 2, as compared to controls) , as presented in figure -1.Wheareas , serum hs-CRP levels expressed a diversity in levels   according to type of diabetes ; type 1 values were significantly lowered (p<

0.05) , but type 2 levels were significantly elevated (p< 0.001) by about 85 % of the control values (figure -2). Serum IL8 levels were highly elevated in type 1 diabetics (figure-3) by > 50% of control values ,while, type2 diabetics exhibited non-significant alteration from that of controls. Considering correlations studies, hs-CRP values showed a significant negative correlations with CCL and serum urea levels in type 1 diabetics (table-2) .On the contrary   non of the studied parameters exhibited a significant Pearson's correlation   with   IL-8 among the studied parameters in diabetic groups.
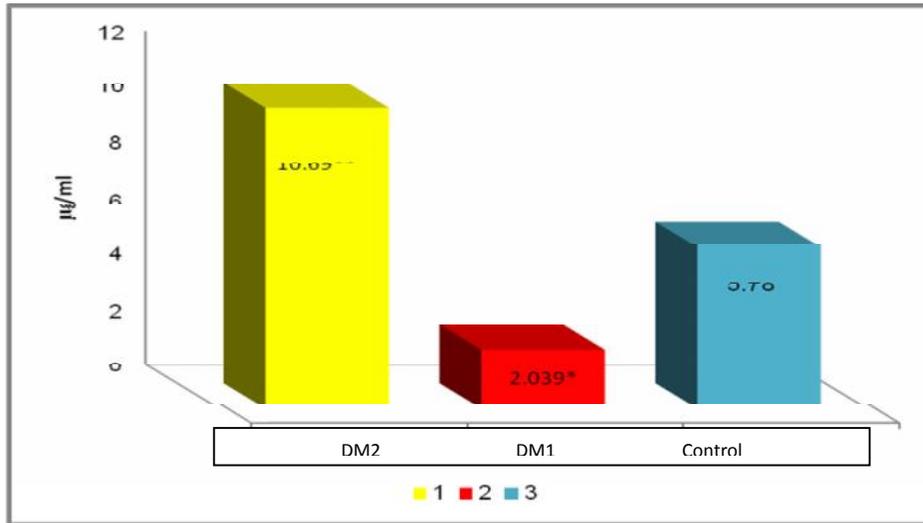

FIGURE 2: Serum hs- C Ractive Protein Levels

Data are presented as mean values, DM2 = Type 2 Diabetes Mellitus, DM1=Type 1 Diabetes Mellitus. *=Significantly different from control (p<0.05) ,**= Significantly different from control (p<0.001).
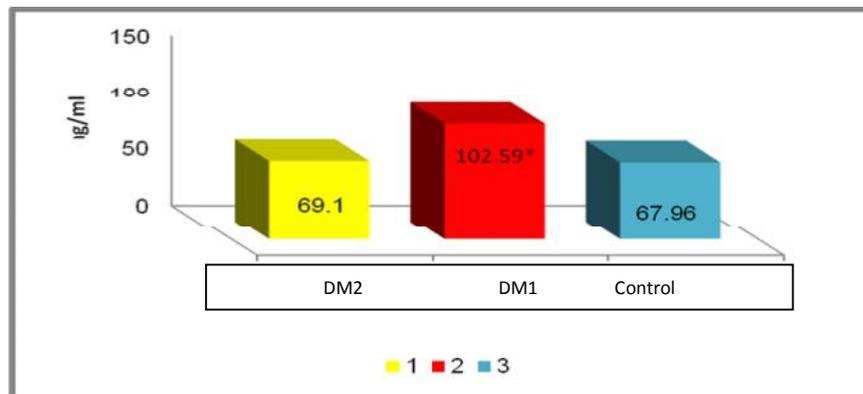

FIGURE-3: Serum Interleukin- 8 levels

Data are presented as mean values   , DM2 = Type 2 Diabetes Mellitus, DM1=Type 1 Diabetes Mellitus. *=Significantly different from control (p<0.05) .

TABLE 2: Pearson's Correlation Values for Highly Sensitive –C Reactive Protein among Studied Groups

Variables	Type 1 Diabetes Mellitus		Type 2 Diabetes Mellitus	
	R	p-value	R	p-value
FSG	0.408	0.083	0.146	0.410
Hb	0.110	0.965	0.079	0.656
CCL	0.116	0.512	0.598*-	.007**0**
Serum Urea	-0.332	0.055	-0.867*	0.0001

FSG=Fasting Serum Glucose, Hb=Hemoglubin , CCL=Creatinine clearance .

*=Significant correlation (p < 0.05) .

TABLE 3: Pearson's Correlation Values for Serum Interleukin -8 values among Studied Groups

Variables	Type 1 Diabetes Mellitus		Type 2 Diabetes Mellitus	
	R	p-value	R	p-value
Hb_{AIC}	0.425	0.070	0.285	0.103
Hb	-0.167	0.496	0.005	0.980
CCL	-0.191	0.062	-0.273	0.118

Hb_{A1C}=Glycated Hemoglubin , Hb=Hemoglobin , CCL=Creatinine clearance .

DISCUSSION

Anemia in diabetic exhibit elevated serum levels of hepcidin (figure-1), which could be related to the previously reported low grade of inflammation of diabetes, as it is regarded to has anti-inflammatory activity [25], that could adversely affect iron metabolism causing development of anemia. Hepcidin acts by binding to ferroportin, the sole known iron exporter, causing its internalization and degradation in the cytosol [26] . This degradation of ferroportin prevents the release of intracellular iron content. Ferroportin is expressed mainly in macrophages and duodenal cells, allowing, respectively, iron recycling and its digestive absorption. Hepcidin may thus inhibit the release of iron from tissue macrophages, leading to iron-restricted erythropoiesis, or from the duodenal cells after its uptake from the digestive lumen, leading to dietary iron deficiency. Thus, hepcidin acts as a "hyposideremic" hormone, aiming at inhibiting iron absorption and reducing the concentration of iron in the blood, which might explain the microcytic type of anemia (lowered MCV values, table-1) presented in type 1 diabetes patients [27] . Low levels of iron in circulation may cause severe dysfunctions (e.g., anemia, hypoxia) while iron overload may be toxic because of its ability to generate reactive oxygen species. Thus, blood iron concentration is finely regulated [28].The variation in type of anemia between type 1 and type 2 diabetics (according to our data) might reflect the alterations in iron availability for erythropioesis in each group , which may contribute to variation in serum levels of CRP (figure-2) through the previously reported adverse effects of iron in diabetic patients through influencing the oxidative balance in those patients, in favour of pro-oxidants over the anti-oxidants [29] . Furthermore, It has been repeatedly reported that iron influences glucose metabolism, which in turn affects iron metabolic pathways. Likewise, body iron stores are positively correlated with serum insulin and blood glucose concentrations [30]. Serum IL-8 levels (figure-3) were not parallel to changes of the other inflammatory mediator, as expected, for serum CRP. C-reactive protein was previously reported as a predictor for micro- & macrovascular complications of diabetes [31]. Although all of the study groups were – ve microalbuminuric , however , the CCL values were significantly reduced (especially in type 2) , indicating the presence of nephrotic microvascular complication without overt renal disease , which might be reflected by lowered CRP levels in type 1 , in comparism between the two groups of diabetes [32] . Despite the negative microalbuminuria of the selected patients for this study, those were presented with decline in CCL values as compared to age matched non diabetic controls . The association between diabetic nephropathy and microalbuminuria is not strong for type 2 diabetes , as reported by Dalla et al , that only 30% of those with microalbominuria demonstrated typical findings of diabetic nephropathy by biopsy from kidney [33] . Whereas , in type 1 diabetics the onset of microalbuminuria during the first four years , found that younger age of diabetes at diagnosis , longer diabetes duration and poorer glycemic control to be positively associated with microalbuminuria [34]. Others, did not related microalbuminuria to type 1 nephropathy, during 5, 10 or 15 years of follow up [35].Consequently the modified renal function could adversely affect erythropoiesis and hence anemia development in those patients [36] . However, it seems that serum levels of IL-8 were associated with elevation in hepcidin levels, indicating a greater role for IL-8 in anemia associated with elevated hepcidin levels , rather than with CRP . Because chronic elevation in glucose level in diabetes , increases monocytes adhesion to endothelial cells [37] , as IL-8 functions as a chemoattractant and is a potent angiogenic factor , IL-8 levels were related to Hb $_{AIC}$ levels in type 1 diabetics – table-3 [4] [38].Besides other anticipated inflammatory mediators in renal dysfunction of diabetes, CRP can potently down regulate e NOS – endothelial nitric oxide synthase - , resulting in deceased basal and stimulated NO release [39]. Furthermore, CRP stimulates ET-1- endothelin -1-and IL-6 release from endothelial cells causing lowered release of essential vasodilators; prostacyclin, shifting the balance towards endothelial dysfunction. Thus, the capillary rarefaction is specific for uremia and renders the myocardium susceptible to ischemic injury [11]. Other potential effects could be related to variations in BMI between type1 and type2 of the studied diabetic groups , regarding adipose tissues as an effective endocrine organ through the effect of specific adipokines such as ; leptin , adiponectin, resistin , etc. that might contribute to total inflammatory state in diabetes [40]. In present study Pearson's correlations analysis indicated that CRP values were correlated negatively , to statistically significant level p<0.01 , to CCL and serum urea values (r= - 0.598 ,- 0.867 , respectively) in type 2 diabetes mellitus , (table - 2).Because CRP was suggested to contribute in kidney dysfunction of diabetics and to be correlated positively with microalbuminuria from the National Health and Nutrition Examination Surveys ,1999 to 2004 [8]. Whereas, Pearson's correlations analysis indicated that IL-8 values were correlated (although not to statistically significant level p<0.07) to HbA1c values r= 0.425 (table - 3). , to be more correlated with glycemic control as indicated by Hb_{A1C} values ,which is the cornerstone for both microvascular and macrovascular complications associated with diabetes , with a similar correlation with

CCL in type 1 diabetes [41]. In conclusion, quantitative determination of IL-8 is more predictive for nephropathy in anemic type1 diabetes than CRP before progression diabetic nephropathy to microalbuminuria. IL-8 in turn reflects the glycemic control to some degree. Meanwhile, serum hepcidin might represent a sensitive marker for anemia in both types of diabetes without overt renal disease.

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