Some Parameters of Inflammation & Oxidative Stress in Relation to the Risk of Type 2 Diabetes Mellitus

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Abstract:

Background: Many clinical trials have indicated that lifestyle modification can delay or prevent the progression to type 2 diabetes in people with impaired glucose tolerance test (IGT). Detection of IGT requires a test which is inconvenient to screen for this condition in clinical practice or in the general population. Therefore, there is a need to search for additional significant risk factors for type 2 diabetes.

Patients &methods: Seventy two adults ≥ 40 years old (36 males and 36 females) were evaluated in this study. They were subjects who performed a brief 75-grams oral glucose tolerance test and were classified as having normal glucose tolerance (NGT group; 24 subjects), impaired glucose tolerance (IGT group; 24 subjects), or diabetes mellitus (DM group; 24 patients).In addition to hematocrit (PCV) and erythrocyte sedimentation rate (ESR), serum level of highly sensitive C-reactive protein (hsCRP) was measured. Also the serum levels of thiobarbituric acid-reactive substances (TBARS), iron, copper and ferric reducing ability of plasma (FRAP) were estimated in addition to erythrocyte glutathione (Ery-GSH) level.

Results: Both IGT group and DM group have a significantly high hsCRP mean level (2.59 ± 1.03 and 2.89 ± 0.90 respectively vs. 1.57 ± 1.40 mg /L in NGT group, P< 0.001), and a significantly decreased FRAP level (939.2 ± 157.4 and 961.5 ± 125.1 respectively vs. 1063 ± 104.5 µmole/L in NGT group, p< 0.01) as well as a significantly high TBARS and a significantly low Ery-GSH level in comparison with NGT group. The positive correlation between hsCRP and TBARS, although was statistically not significant, showed a step-wise increment from NGT, to IGT and to DM group (r = 0.01, 0.14 and 0.23 respectively).

Conclusions:IGT is associated with a state of low-grade inflammation and oxidative stress. Serum levels of hsCRP as a marker of inflammation and TBARS as a marker of oxidative stress may serve for identifying people at high risk for developing type 2 diabetes. Such people will be important targets for programs that are designed to prevent diabetes.

Key words: Impaired glucose tolerance, Inflammation, Oxidative stress, hsCRP, TBARS

Introduction:

The prevalence of diabetes mellitus (DM) is increasing worldwide[1]. .Type 2 diabetes mellitus (T2DM) comprises about 90 % of all diagnosed cases of DM[2]. Diabetes is costly to both the affected individual and to society and prevention of T2DM would result in significant public health benefits, including lower rates of cardiovascular disease (CVD), renal failure, blindness, and premature mortality within the population[3].

Previous studies have identified physical and biochemical variables that are associated with subsequent development of T2DM. These variables included older age, obesity (especially abdominal obesity), physical inactivity, history of gestational diabetes mellitus, high fasting insulin level and impaired glucose tolerance (IGT)[4].

The term impaired glucose tolerance (IGT) indicates a metabolic stage intermediate between normal glucose homeostasis and diabetes[5]. Subjects with IGT are at a significant risk for diabetes[6] and are sometimes referred to as prediabetics although diabetes will not necessarily

develop in all those with IGT[7]. In an analysis of six prospective studies, the risk of developing diabetes was found to be approximately 3.6 to 8.7 percent per year in subjects with IGT[6]. Subjects with IGT are thus good targets for programs that are designed to prevent diabetes[8].
Detection of IGT requires a test which is inconvenient to screen for this condition in clinical practice or in the general population. There is a need to search for additional risk factors for T2DM which may be more convenient or more efficient in identifying subjects who need to be targeted by preventive programs[9].
Many studies have reported an association between inflammation and overt T2DM . This was first demonstrated as a higher plasma level of sialic acid and C-reactive protein in diabetic individuals[10]. Low grade Inflammation was later speculated to be a prelude to T2DM [11].
Plasma from diabetic patients has long been shown to contain high levels of lipid peroxides[12] and diabetic patients are often stated to be under an oxidative stress[13] but it is not clear whether this stress is a cause or effect or is an association to diabetic complications such as vascular complications[14]. Oxidative stress has even been suggested to be the unifying link between the various molecular disorders in diabetes[15].

The aim was to study some parameters of inflammation and oxidative stress in subjects with IGT which may contribute to identification of subjects at high risk for T2DM..

Patients and Methods:

Settings: This study was conducted at Department of medical biochemistry, College of medicine and National diabetes center, University of Al-Mustnasiriya, Baghdad, Iraq, from April 2004 to May 2005. Seventy- two subjects (36 males and 36 females), whose age should be ≥ 40 years, were randomly selected from those referred for performing a brief oral glucose tolerance test (OGTT) in the diabetes center. They were either subjects who were suspected to have diabetes mellitus and referred for checking their glycemic state or subjects visiting the specialist clinics of internal medicine for certain complaints and were defined to have clinical risk factors for diabetes, particularly obesity, thus were referred for checking their glycemic state. The latter was an opportunistic type of screening [7].

Subjects with a concurrent acute illness or with a major liver, thyroid or other endocrine diseases were excluded. Weight and height were reported in order to calculate body mass index (BMI), Waist: hip ratio was used as an indicator for central obesity. The study protocol was approved by local institutional scientific committee and all subjects gave informed consent.

Study subjects were classified according to the result of their 2-hour serum glucose level (2hr-S.G) in the OGTT into three groups[5]. Those with 2hr-S.G less than 140 mg/dl were assigned as normal glucose tolerance (NGT group), those with 2hr-S.G between 140 and 200 mg/dl as impaired glucose tolerance (IGT group), and those with 2hr-S.G ≥ 200 mg/dl as diabetics (DM group).

Laboratory investigations:

Specimen collection: The subjects were instructed about the standard preparations for a brief OGTT at the time of giving them an appointment for the test[7]. Venous blood was obtained at the start of the test for estimation of fasting serum glucose level, followed after 2 hours of giving 75 grams oral glucose load by aspiration of 2 ml venous blood for estimation of the 2-hour serum glucose level. Fasting blood was divided into the following portions: packed cell volume (PCV) capillary tube , sodium –citrate solution tube for erythrocyte sedimentation rate (ESR), and acid –citrate –dextrose tube for erythrocyte glutathione. Fasting blood collected in a plain tube was used for ferric reducing ability (FRAP) test, highly-sensitive C-reactive protein (hsCRP), thiobarbituric acid-reactive substances (TBARS) test, iron (Fe) and copper (Cu). Serum was used for measuring FRAP test during few hours. The rest of the serum was divided into aliquots and stored at –20 Cº until analysis.

Glucose estimation: Serum glucose level was determined spectrophotometrically by an enzymatic method.

Oxidative stress markers: These included serum thiobarbituric acid-reactive substances (TBARS)[16], erythrocyte glutathione(17), ferric reducing ability of plasma (FRAP)[18], serum Iron [19], and serum copper[20].

Inflammatory markers: Serum highly sensitive C-reactive protein (hsCRP) was determined quantitatively using hsCRP ELISA kit purchased from DRG international Inc., USA. Measurement was performed with a generous help in the unit of immunological assays, Al-Yarmouk hospital teaching laboratories. Erythrocyte sedimentation rate was measured by the standard Westergreen method.

Statistical analysis:

Data were presented in simple statistical measures of number, percentage, mean and standard deviation. Statistical analysis was done by using Student's t- test and simple linear correlation. A probability value ($p<0.05$) was considered to be statistically significant.

Result:

The mean age of females of IGT group and the mean age of both males and females of DM group are significantly higher than that of NGT group (Table 1). The mean values of body mass indices (BMI) of study groups are comparable. Assessment of the waist / hip (w/h) ratio shows a significantly higher mean value in DM group than in NGT group. Although the mean value of w/h ratio in IGT group is higher than that of NGT group, it does not reach to the level of significance.

Table (2) shows the serum glucose levels of DM group as well as of IGT group in comparison with that of NGT group. Glycemic state is demonstrated in fasting state and at 2 hours of OGTT. Detailed analysis has revealed that three out of twenty four diabetic patients have had within normal range a fasting glucose level indicating that some suspected diabetics in the study were well controlled.

A significantly higher hematocrit value is observed in DM group than in NGT group, which amounted about 5 % increment (42.5 ± 6.59 vs. 37.33 ± 6.37, $p = 0.01$) (Table 3).

The results obtained with ESR measurements shows that a significantly higher ESR mean value is observed only in males of IGT group in comparison with males in NGT group (Table 3). Diabetic males are also having a significantly higher ESR mean value in comparison with that of NGT group.

A significantly higher hsCRP mean value is observed in both IGT group and DM group. Again, males rather than females are having a significantly high hsCRP mean value (Table 3).

Diabetic patients show a significantly high serum thiobarbituric acid reactive substances (TBARS) mean level which approximates four folds increment than that of NGT group (10.18 ± 6.89 vs. 2.76 ± 1.31, $p < 0.001$) (Table 4).

Subjects with impaired glucose tolerance are also having a significantly high TBARS mean level but the increment is less than that observed in diabetics, and is not approximating two folds (4.55 ± 2.57 vs. 2.76 ± 1.31, $p <0.01$) (Table 4).

Total antioxidant activity of plasma in terms of FRAP is significantly decreased in both IGT group and DM group (Table 4). Interestingly, we observe that FRAP activity is lower in subjects with impaired glucose tolerance than in diabetic patients (Table 4)

Cellular antioxidant activity in terms of erythrocyte glutathione is also decreased in diabetic patients (DM group) and in impaired glucose tolerance subjects (IGT group). The mean erythrocyte glutathione levels are 69.85 ± 26.26 mg/dl ($p< 0.01$) and 70.88 ± 27.84 mg/dl ($p<0.01$) in (DM group) and (IGT group) respectively in comparison with a control value of 89.71 ± 22.48 mg / dl in NGT group (Table 4).

A significantly high serum iron level is observed in diabetic patients. It accounts 106.7 ± 36.75 µg /dl vs. 81.65 ± 34.23 µg /dl of controls (P< 0.05) (Table 4). The mean value of serum iron related to IGT group does not reach the level of a significant difference from that NGT group.

Although the mean serum copper level of patients belonging to DM group is higher than in controls NGT group, this does not reach to the level of significance.

The hsCRP values showed a significant direct correlation with ESR values in all study groups (Table 5). In study of the correlations with other hematological, inflammatory and oxidative stress markers, two points were observed. First, the correlation between hsCRP and TBARS showed a stepwise increase from NGT group to IGT group and to DM group (r = 0.01, 0.14 and 0.23 respectively) (Table 5). Second, the correlation between hsCRP and erythrocyte-GSH was negative or inverse although this was statistically significant only in the DM group and in the NGT group.

.Table 1 :Some clinical characteristics of study groups

Variable	Normal glucose tolerance (NGT)			Impaired glucose tolerance (IGT)			Diabetes Mellitus (DM)		
	Male (n=12)	Female (n=12)	Total (n=24)	Male (n=12)	Female (n=12)	Total (n=24)	Male (n=12)	Female (n=12)	**Total (n=24)**
Age (yr.) (Mean ± SD)	48.92 ±9.97	45.17 ±4.32	47.04 ± 7.75	47.04 ±7.78	50.33 ± 6.91*	48.75 ± 7.37	57.92* ±9.79	52.75* ±10.00	**55.33** * **±0.01**
Body mass index (BMI) (Mean ± SD)	29.89 ± 4.61	29.89 ±4.88	29.89 ±4.64	28.83 ±4.77	32.27 ±4.87	31.05 ± 5.23	29.46 ±3.12	31.51 ± 4.88	**30.49 ± 4.15**
Waist/ hip ratio (W/h) (Mean ± SD)	**0.94± 0.05**	**0.96 ±0.06**	**0.95 ±0.07**	**0.99 ±0.02**	**0.97 ± 0.07**	**0.98 ± 0.05**	**1.00 ± 0.04**	**0.98 ± 0.08**	**1.00* ± 0.06**

{* *p*<0.05, ** *p* <0.01} in comparison with corresponding values of (NGT group)

Table 2: Assessment of glycemic state of study group

Variable	Normal glucose tolerance(NGT)			Impaired glucose tolerance (IGT)			Diabetes Mellitus (DM)		
	Male (n=12)	Female (n=12)	Total (n=24)	Male (n=12)	Female (n=12)	Total (n=24)	Male (n=12)	Female (n=12)	Total (n=24)
Fasting serum glucose (mg/dl)	91.08 ±15.58	89.92 ±13..24	90.50 ±14.15	95.67 ±13.23	99.92 ±13.51	97.79 ±13.26	151.25* ±32.33	148.67* ±56.11	149.96* ±44.80
hOGTT (mg/dl)	7.25 ±15..32	117 ± 16.93	107.13 ±18.74	182.58* ±18.67	153.33* ±32.08	167.96* ±29.70	297.67* ±41.22	252* ±38.58	274.83* ±45.4

*** *p*<0.05 in comparison with corresponding values of NGT group**

Table 3 :Inflammatory and hematological variables of study groups

Variable	Normal glucose tolerance (NGT)			Impaired glucose tolerance (IGT)			Diabetes Mellitus (DM)		
	Male (n=12)	Female (n=12)	Total (n=24)	Male (n=12)	Female (n=12)	Total (n=24)	Male (n=12)	Female (n=12)	**Total (n=24)**
Hematocrit (%)	40±5.02	34.7 ±6.66	37.39± 6.39	39.25 ±3.25	37.58±4.27	38.42± 4.06	46.17± 6.58***	38.83± 4.22	42.45± 6.58**
ESR (mm/1sthr)	7.75 ±4.25	27.67 ±20.1	17.71± 17.52	23.83± 14.28**	12.25 ±9.96***	18.04± 13.42	18.5±16.18***	30.67± 12.73	**24.58± 15.56**
hsCRP (mg/L)	1.07±1.02	2.04± 1.60	1.57±1.40	2.34±1.22***	2.84± 0.79	2.59±1.03**	2.76 ±0.76*	3.01 ±1.04	2.89±0.90*

* ***p*** **<0.05, ** *p* <0.01 , *** *p* <0.001 in comparison with corresponding values of (NGT group)**

Table 4 :Variables that are related to oxidative stress in study groups

Variable	Normal glucose tolerance (NGT)			Impaired Glucose tolerance (IGT)			Diabetes Mellitus (DM)		
	Male n= 12	Female N= 12	Total n=24	Male n= 12	Female n= 12	Total n=24	Male n= 12	Female n= 12	**Total n=24**
Thiobarbituric acid-reactive substances (µmol /L)	2.91 ± 1.32	2.63 ±1.34	2.76 ±1.31	3.60 ±1.59	5.50 ±3.04**	4.55** ±2.57	9.60*** ±4.51	10.79** ±8.53	**10.18*** ±6.89**
Ferric reducing ability of plasma (µmol/L)	1112.6 ±152.1	998.4 ±150.1	1063 ±104.5	968* ±195.5	910.4 ±109.5	939.2** ±157.7	990.2* ±135	932.9 ±112.6	**961.5* ±125.1**
Erythrocyte-glutathione (mg/dl)	104.3 ±16.6	75.08 ±17.70	89.71 ±22.48	67.08*** ±14.82	74.67 ±37	70.88** ±27.84	78.98* ± 31.20	60.73 ±17.78	**69.85** ±26.26**
Serum iron (µg/dl)	89.63 ± 38.58	73.68 ±28.68	81.65 ±34.23	92 ± 46.52	89.83 ±21.19	90.92 ± 35.37	107.33 ± 10.10	106 ± 49.99	**106.7* ±36.76**
Serum copper (µg/dl)	77.3 ± 29.32	103 ± 59.67	90.58 ± 47.94	84 ±40.18	74.33 ±40.18	79.17 ± 34.98	110.39* ±35.66	111.82 ±33.90	111.10 ±34.04

{* *p*<0.05, ** *p* <0.01 , * *p* <0.001 } in comparison with corresponding values of (NGT group)**

Table 5 :Correlation between hsCRP and other inflammatory, hematological and oxidative stress markers

hs-CRP	Normal glucose tolerance (NGT)	Impaired glucose tolerance (IGT)	Diabetes mellitus (DM)
ESR	0.78*	0.66*	**0.85***
PCV	- 0.30	- 0.17	**0.13**
TBARS	0.01	0.14	**0.23**
FRAP	- 0.36	- 0.09	**- 0.15**
Ery-GSH	- 0.44**	- 0.10	**- 0.44****
Fe	- 0.21	- 0.18	**0.24**
Cu	**0.019**	**- 0.12**	**- 0.02**

{* *p*<0.05, ** *p* <0.01 }

Discussion:

Screening to detect subjects with IGT during health care visits in people aged over 40 years, particularly those with a BMI of 25 kg/m2 or more, has already been recommended[21]. More practical or convenient tests that can efficiently identify subjects at high risk for diabetes will be widely accepted in clinical and public health settings.

Our study has revealed a significantly high hsCRP levels in both IGT and DM groups. Moreover, the mean level of hsCRP in IGT group was significantly higher than in NGT group but lower than in DM group. This graded increment in hsCRP may help prediction for future diabetes although the cut-off point of hsCRP cannot be determined. Our results are in line with prospective studies on apparently healthy middle-aged populations[22,23], middle-aged women[24] and elderly subjects (≥ 65 years)[25] in which serum CRP level was found to be determinant of risk for development of T2DM. Moreover, in a retrospective study there was a strong and graded association of CRP level with incident DM independent of established risk factors[26].

The above findings are in support of the hypothesis that IGT and DM have an inflammatory aspect, but the causal pathway is not clear. Does DM or higher serum glucose level brings about inflammation?[27] alternatively, it is possible that inflammation could be the primary disorder that leads to insulin resistance[22,28,29]. Also it is possible that inflammation and DM arise from another, as of yet, unidentified "common" genetic antecedent.[30].

The finding of a significant positive correlation between hsCRP and ESR in all study groups is most probably related to the findings in other studies that the plasma level of fibrinogen, an another acute phase reactant and a major determinant of ESR, is elevated in cases of insulin resistance and type 2 diabetes mellitus[22].

An evidence for oxidative stress in our work included a significant high serum TBARS and a significant low erythrocyte-GSH as well as a significant low serum FRAP level in both IGT and DM groups. This evidence is consistent with some previous works in regard to higher serum TBARS level[31] and higher blood glutathione level in IGT subjects[32]. These results support the idea that oxidative stress may be an early event in the natural history of diabetes[33].

It has been reported that oxidative stress can activate multiple serine kinase cascades including IKKB/ NF-kB that can impair insulin action and induce hyperglycemia[34,35]. FRAP assay which was suggested to investigate effect of disease on antioxidant status[18] did not show superiority to TBARS test in regard to the change of values as a percentage.

In both IGT and DM groups there was a significant increase in serum TBARS in addition to a significant increase in serum hsCRP . The positive correlation between TBARS and hsCRP , although was statistically not significant showed a stepwise increment when we ascend from normal,- to IGT group, ending to DM group. This may refer to an implication of oxidative stress in promoting a low – grade inflammation in IGT subjects and DM patients. In this regard, it has been found that

glutathione, a powerful antioxidant, completely prevented a cytokine increase that is induced by oscillatory hyperglycemia in healthy and IGT subjects[27]. Additionally, hyperglycemia has been shown to induce oxidative stress in adipose tissue which causes enhanced activation of inflammatory pathways[35].

The results reported in this work showed a significant rise in the mean hematocrit level in DM group but not in IGT group. It has been reported previously that hematocrit level is increased in patients with established diabetes as one of hematorheological abnormalities that may enhance the risk of vascular disease[36]. Still, in another study there was an association between a higher hematocrit level and an increased risk of type 2 diabetes even in subjects with hematocrit measurements that are within the normal range[37]. This makes one expect a somewhat higher hematocrit level in IGT group. However, our finding may be explained by our selection of newly diagnosed dysglycemic patients while a higher hematocrit level may be associated with a long standing dysglycemia including IGT state. Thus, a higher hematocrit level may be an epiphenomenon rather than a cause in dysglycemia.

Our study has detected a higher serum iron level in IGT and DM groups although the increase was statistically significant only in DM group. This finding may have important implications to the pathogenesis and complications of diabetes. This increase in iron, acting as a Fenton reagent, might be a contributor to oxidative stress in diabetes[38]. The same may be applied on the findings that serum copper level in our work is higher in diabetic patients than in NGT subjects although the increase has reached statistical significance only in male diabetics. In some studies, high serum copper has been incriminated in oxidative damage[39].

In conclusion, IGT is associated with a state of low-grade inflammation and oxidative stress. Serum levels of hsCRP as a marker of inflammation and TBARS as a marker of oxidative stress may serve for identifying people at high risk for developing type 2 diabetes. Such people will be important targets for programs that are designed to prevent diabetes.

Conclusions:

In conclusion, IGT is associated with a state of low-grade inflammation and oxidative stress. Serum levels of hsCRP as a marker of inflammation and TBARS as a marker of oxidative stress may serve for identifying people at high risk for developing type 2 diabetes. Such people will be important targets for programs that are designed to prevent diabetes.

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