The Relevance of Glycosylated Hemoglobin with Oxidative Stress in Insulin Resistant Type 2 Diabetes Mellitus

Majid Kadhum Hussain*, Dhafera Jaffer Abed-Ali**, Makarim Hisham Mohammed**

*University of Kufa, College of Medicine Department of Biochemistry

** University of Kufa, College of Education for Women, Department of Biology

Abstract

The present study was conducted to evaluate the relevance of glycosylated hemoglobin with oxidative stress in insulin resistant type 2 diabetes mellitus. To achieve this aim, 93 type 2 diabetic patients of ages 38-84 years were recruited. In addition, 19 apparently healthy individuals with ages 30-60 years, were enrolled as a control group.The concentration of fasting blood glucose (FBG), triglyceride (TG), insulin, glycosylated hemoglobin (HbA_{1c}), malodialdehyde (MDA) and glutathione-S-transferase (GST) were measured in sera of patient and the control groups. Fasting blood glucose and triglyceride levels were estimated by spectrophotometeric methods using enzymatic procedures. Insulin level was estimated by enzyme linked immunosorbant assay (ELISA) method. HbA_{1c} level was determined by an ion exchange chromatographic method, while MDA and GST levels were measured by spectrophotometeric procedures. Insulin resistance was evaluated by four methods. They include homeostatic model assessment (HOMA), quantitative insulin check index (QUIKI), McAulye (McA), and fasting insulin (FI) methods. Insulin resistance was found in 79 (84.9%), 63 (73.4%), 52 (55.9%) and 35 (37%) out of the 93 diabetic patients by HOMA, QUICKi, McA and FI methods respectively. Type 2 insulin resistant (79) diabetic patients (IRP) that obtained through the HOMA method were assessed for the HbA_{1c}, MDA and GST levels in comparison to the insulin sensitive patients (ISP) as well as to the control group. The analysis of the data revealed a significant increase ($p<0.001$) of HbA_{1c} levels in the IRP group when compared with those of
the control group, while the insulin sensitive group (ISP) couldn't show significant variation when compared with those of the IRP. The data of MDA failed to give significant variation. However, a significant elevation of GST concentration were observed in the IRP group with respect to those of the control group ($p<0.005$). On the other hand significant variations could not be obtained among the ISP and IRP. These data suggested that oxidative stress changes are independent on insulin resistance in type 2 diabetes mellitus.

الخلاصة

تم تصميم الدراسة الحالية للتحري عن التغيرات الحاصلة في الإجهاد التاكسدي, والهيموكلوبين السكري المترافقة مع مقاومة الأنسولين في المرضى من النمط الثاني من داء السكري. ولتحقيق هذا الهدف تم اختيار 93 مريضا بالنمط الثاني من داء السكري بأعمار تتراوح بين 38-84 سنة, بالإضافة إلى 19 شخصا بصحة جيدة حيث ان أعمارهم كانت بين 30-60 سنة كمجموعة مراقبة. وتم قياس كلا من التراكيز الصيامية (للكلوكوز, ثلاثي الكليسيرايدات , والأنسولين, الهيموكلوبين السكري, المالون ثنائي الالديهايد والكلوتاثايون الناقل للكبريت) للمرضى ومجموعة السيطرة.تم استخدام الطرق الإنزيمية وطرق التحليل الطيفي لقياس تراكيز الكلوكوز وثلاثي الكيلسيريدايت. تم قياس مستوى الأنسولين بواسطة طريقة (ELISA), فضلا عن تحدد مستوى الأنسولين بتقنية كروموتوكرافيا المبادل ألايوني, بينما استخدمت طرق التحليل الطيفي لقياس مستوى المالون ثنائي الالديهايد و الكلوتاثايون الناقل للكبريت.تم استخدام اربع طرق لتحديد عدم الاستجابة للأنسولين وهي HOMA, QUICKI, McA, FI.عدم الاستجابة للأنسولين كمايلي: 79 من اصل 93 والتي تمثل 84.9 % , و 63 من 93 والتي تمثل 73.4 %, و 52 من 93 والتي تمثل 55.9 % , و 35 من 93 والتي تمثل 37.6 % من

المرضى لم يستجيبوا للأنسولين بصورة طبيعية اعتمادا على طريق HOMA, QUICKI, McA, FI على التوالي. إن المرضى المقاومين للأنسولين (٧٩) من النوع الثاني من داء السكري بطريقة ال HOMA هم الذين اخذوا بنظر الاعتبار في إجراء الحسابات وتبيان الاختلافات في مستوى المقاييس بالمقارنة مع مجموعة المرضى الحساسة للانسولين ومجموعة المراقبة . إن تحليل المعطيات قد أدى إلى وجود ارتفاع معتد به إحصائيا لمقياس الهيموكلوبين السكري في مجموعة المرضى المقاومين للأنسولين ($p<0.001$) بالمقارنة مع مجموعة المراقبة, بينما لم تظهر مجموعة المرضى الحساسة للأنسولين تغاير عند مقارنتها بمجموعة المرضى المقاومين للأنسولين. فشلت معطيات المالون ثنائي الالديهايد في إظهار أي تغاير معنوي. وقد لوحظ ارتفاع معتد به إحصائيا لتركيز الكلوتاثايون الناقل للكبريت للمجموعة المقاومة للأنسولين عند مقارنتها بمجموعة المراقبة($p<0.005$) , من جانب أخر لم تظهر تغايرات معنوية بين المجموعة الحساسة للأنسولين والمجموعة المقاومة للأنسولين.اقترحت تلك المعطيات إن تغايرات الإجهاد التاكسدي غير معتمدة على مقاومة الأنسولين في النوع الثاني من داء السكري.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose level (hyperglycemia) resulting from defects in insulin secretion, insulin action, or both. The term diabetes mellitus is derived from the Greek words meaning "to run through" (Kojo, 2004) Hyperglycemia is a widely known cause of enhanced plasma free radical concentrations. Free radical production and consequently the oxidative stress may be induced by hyperglycemia via at least four different routes which include increased glycolysis, intercellular activation of sorbitol (polyol) pathway, autoxidation of glucose and non enzymatic protein glycation (Ahmed, 2005). Insulin resistance (IR) is a physiological condition where the natural hormone insulin, becomes less effective at lowering blood sugars. The resulting increase in blood glucose may raise levels outside the normal range and cause adverse health effects, depending on dietary conditions. Certain cell types such as fat and muscle cells require insulin glucose uptake, when these cells fail to respond adequately to circulating insulin, blood glucose levels rise (Murano et al., 2008). The subnormal biological response could be due to the inability of plasma insulin to bind to its receptor or the presence of a post-receptor binding defect (Weisberg et al., 2003). IR is associated with a number of diseases including obesity, metabolic syndrome, T2DM, polycystic ovary syndrome and chronic infection (Scott et al., 2005). Insulin resistance in muscle and fat cells reduces glucose uptake, whereas insulin resistance in liver cells results in reduced glycogen synthesis and storage and a failure to suppress glucose production and release into the blood (Kahn and Flier, 2000). The term glycation is the nonenzymatic reactions that link a sugar to a protein or peptide. The product of glycation is a glycoprotein, or, in the special case of the reaction with hemoglobin, glycohemoglobin, or glycated hemoglobins. In a normal person glucose circulates in the blood. The erythrocytes are freely permeable to glucose and the concentration in the cell is approximately the same as in the plasma. When glucose levels are elevated in plasma, they are proportionately elevated in the erythrocytes (World Health Organization, 2002). Different forms of glycosylated hemoglobin have been identified. HbA1c is the product of heamoglobin reaction with glucose molecule. It is used to assess the glycaemic control for the last 6-8 months (World Health Organization, 2002). Malondialdehyde (MDA) is a three-carbon, low-molecular weight aldehyde, produced as a by product of the lipid peroxidation. The latter is enhanced when the level of free radicals and elevates (Sanocka and Kurpisz, 2004). To protect the cells and organ systems of the body against the adverse effects of oxidative stress, humans have evolved a highly sophisticated and complex antioxidant protection systems. They are classified into two categories (Mittler et al., 2004),

enzymatic and non enzymatic antioxidants. One of the enzymatic antioxidants is glutathione-S-transferase (GST) which is involved in the metabolism of divers endo- and xenobiotics. It protects the cells against Oxidative Stress (Wang, 2005). GST is a dimeric enzyme catalyzes the conjugation of GSH to a variety of electrophiles including xenobiotic chemicals and endogenous toxic substances such as ROOH (Seufi et al., 2009). The relevance of oxidative stress with glycosylated haemoglobin is not clear, so that the current investigation is conducted to explore such relationship.

Materials and Methods

The study was conducted on randomly selected 94 type 2 diabetic patients (33 male and 61 female) attending the diabetes mellitus center in Al-Sadder Teaching Hospital in Najaf province. It was carried out from February 2011 to July 2011 in the laboratory of research/Department of Biology in College of Education for Women and the Department of Biochemistry in College of Medicine/University of Kufa. The age of patients was 58.02 ±10.11 y with a range of 38-84 y.

Diabetes mellitus was diagnosed by consultant doctors. The information of patients were obtained through a questionnaire consisted of the name, sex, age, weight, height, duration of the disease, complications and other diseases. Patients with renal dysfunction, heart diseases, who were on drugs affect oxidative stress, i.e, antioxidants and anti-hyperlipidemic agents were excluded from the current investigation. The study was carried out

A group of 20 apparently healthy subjects (9 males and 11 females) were included as a control group. Their ages were 42.20±7.38 y with a range of 30-60 y. During the dialogue with the volunteers of the control group, they seemed to be free from health problems. The information of the control group were obtained as that of the patients.

Disposable syringes and needles were used for blood collection by vein puncture. Venous fasting blood samples (5 mL) were collected from the patients and healthy volunteers after an overnight fasting. One milliliter of blood was transferred to a tube contained EDTA, it was used for the estimation of HbA1c concentration. The remaining blood sample was transferred to a plastic tube; it was left to clot at 37 °C for 15 min. Blood samples were centrifuged at 3000 xg for 15 min. The sera were separated into three aliquots and stored at -17 °C prior to the determination of the biochemical parameters. Glycosylated hemoglobin concentration was determined by using the kit from Stanbio Laboratory data (2011). Fasting blood glucose concentration was measured by Bablock method (1988).Triglyceride level was estimated by Fossati and Prencipe method (1982). The level of fasting insulin was determined by a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle (DeFronzo, 1999). Estimation of insulin resistance was evaluated by four methods, i.e, Fasting insulin concentration (FI), Homeostasis Model Assessment (HOMA) = [glucose (in mmole/L) * insulin (in microU/mL)] / 22.5, Quantitative Insulin Sensitivity Check Index (QUICKI) = 1/[log glucose (in mg/dL) + log insulin (in microU/L)], McAuley's index (McA) = exp [2.63 - 0.28 ln (insulin [in microU /mL]) - 0.31 ln (triglycerides [in mmole/L])](Sathiyapriya et al., 2007). Patients were considered as insulin resistant when:

- FI $\geq$ 12 µU/L.
- HOMA $\geq$ 2.5
- QUICKI $\leq$ 0.33
- McA $\leq$ 5.8

The level of malondialdehyde concentration (MDA) was determined by a method described by Guidet and Shah (1989). Estimation of serum glutathione-S-transferase activity was acheived by Habig

et. al., (1974). The data were expressed as mean ± SD unless otherwise stated. Statistical analyses were carried out by using student t-test, ANOVA and Pearson`s correlation analysis through the MINITAB Student-Unititled program. Significant difference was considered when the $P < 0.05$.

Results and Discussion

Significant elevations of HbA_{1c} ($P<0.001$), insulin ($P<0.001$), FBG ($P<0.001$), Tg ($P<0.001$) and GST ($P<0.002$) levels were indicated in the group of patients when compared with those of the control group. However, MDA levels showed an insignificant decrease during a comparable assessment (Table 1)

The increased HbA1c levels reflect the poor metabolic control of diabetic patients (Ikekpeazu et al., 2011). This increase is directly proportional to the blood glucose level at the last 6-8 weeks (Moussa and Romanlan, 2008). Higher Levels of glucose in the blood contributes to more binding and consequent high concentration of glycosylated hemoglobin (Kilpatrick, 2000).

The elevated levels of insulin in diabetic patients may be due to the derangement of insulin secretion that may take place during the course of type 2 diabetes, as the pancreas attempts to compensate for the elevated fasting plasma glucose (FPG) concentration. However, as the FPG concentration continues to rise, β-cells are no longer able to sustain their increase rate of insulin secretion and as insulin secretion declines, impaired glucose tolerance (IGT) and eventually overt type 2 diabetes will ensue (Ralph and DeFronzo, 2004 Increased hepatic glucose production (HGP) and decreased muscle glucose uptake further contributes to the state of hyperglycemia. It places further stress on the β-cells and establishes a negative back-loop in which the metabolic control decompensate (glucotoxicity and lipotoxicity) (Adbul- Ghani et al., 2006; Defronzo, 2009). Consequently this contributes to the β-cells failure and worsening insulin resistance. These results are in agreement with those reported by Kalaivanam et al. (2006) Lebovitz (2006) and Goud et al. (2011). In diabetes mellitus, the persistent hyperglycemia may elevate the reactive oxygen species (ROS) concentration from glucose auto-oxidation and protein glycosylation. This elevation may increase the lipid peroxidation with consequent raised levels of MDA (Kalaivanam, 2006). Such observation was not apparent in the present investigation since MDA level changes seemed to be insignificant. It may be owing to the active compensatory antioxidant system represented by the antioxidant enzymes. This hypothesis may be supported by the significant increase of GST activity in the recruited diabetic patients relative to those of the control group. These results are in consistence with those verified by Velazquez et al., (1991). However, they are in disagreement with those reported by Benrebai et al. (2008) and Lalitha etal.(2010)The evaluation of insulin resistance revealed that 79 out of 93 patients (84.9%) were found to be insulin resistant by HOMA, 73 out of 93 patients (78.49%) were found to be insulin resistant by QUICKI, 52 out of 93 patients (55.91%) were found to be insulin resistant by McA and 35 out of 93 patients (37.63%) were found to be insulin resistant by FI (Fig.1). However, one healthy person exhibited insulin resistance through the assessment by HOMA, QUICKI and FI methods, but not by McA method (Table 2).This patient was excluded from the control group.

To select insulin resistant patients as accurate as possible in the present investigation, the four indirect methods of evaluation of insulin resistance were examined. The data exhibited high rate of insulin resistance through the HOMA and QUICKI methods (84.9% and 78.49% of diabetics were insulin resistant

respectively) and low rate with McA and FI methods (55.91% and 37.63% of diabetics were insulin resistant respectively). Thus the HOMA method was implicated for the selection of insulin resistant diabetics. Two factors have strongly led us for the HOMA implication. The first is the wide use of HOMA in the previous works mentioned in literatures (Young et al., 2006; Jin and Pan, 2007). The second is that 79% of the enrolled patients were overweight or obese. Insulin resistance is a serious mechanism involved in the pathogenesis of type 2 diabetes mellitus in particular those of abnormal weight. Thus the data of the HOMA method was highly suggestive to be used for selection of insulin resistant type 2 diabetics, therefore 79 out of 93 patients were categorized as insulin resistant diabetics The results of the current study are in agreement with those of Amato et al. (2006) and Erus et al. (2007) in regards to the data of HOMA methods. However, they were inconsistent those reported by McAuley et al. (2001) and Lukshmy, et al. (2006). The most satisfactory reasons for the difference may be the patient's status and the number of the enrolled diabetics. Some authors have mentioned that FI method is also an alternative rational for the evaluation of insulin resistance and could demonstrate comparable results for those of other indirect methods. The present study is inconsistent with such findings and determination of fasting insulin level seemed to be inappropriate for the prediction of insulin resistance accurately **(**Lukshmy et al., 2006) The insulin resistant patients (IRP) contained 79 patients, while the group of insulin sensitive patients (ISP) consisted of 14 patients. The two groups of insulin sensitive and insulin resistant type 2 diabetic patients as well as the insulin sensitive healthy subjects were compared together for the difference of the glycosylated hemoglobin (HbA_{1c}),

malondialdehyde (MDA) and glutathione-S-transferase (GST) levels. The results showed a significant elevation ($p<0.001$) of HbA_{1c} in the IRP when compared with those of the control group, such difference was not demonstrated with respect to those of ISP. The result of MDA estimation failed to give significant variation. On the other hand, data of GST activity pointed out a significant increase ($p<0.005$) in the IRP when compared with those of the control group. Such difference could not be obtained for IRP with ISP (Table 3). Type 2 diabetes mellitus is partially characterized by elevated fasting serum glucose (FSG), insulin concentration (in most cases), the percentage of HbA_{1c} and decreased insulin sensitivity (Lebovitz, 2006). Insulin resistance is frequently brought on by obesity or being overweight which results in reduction of insulin receptors and impaired post-insulin binding signaling transduction mechanisms (Liese et al., 2005). The response of the pancreas to insulin insensitivity is to increase the blood serum concentration of insulin (Rewers et al., 2004). However, this rise in insulin levels seldom compensates completely for the insulin insensitivity and consequently serum glucose concentrations rise (Cefalu, 2001). When blood glucose concentration rises, HbA_{1c} level will elevate as there is an increased ratio of glucose to hemoglobin concentration, allowing the glycosylation process to occur at a high rate (Mentink et al., 2006).This is the most likely cause of HbA_{1c} increase in IRP when compared with those of the control group. However the insignificant difference between IRP and ISP indicates that HbA_{1c} formation is independent on insulin resistance. The insignificant change of MDA and GST levels in IRP in comparison to the ISP suggests that oxidative stress alteration is independent on insulin resistance in type 2 diabetes mellitus. This observation may be attributed to the racial adaptation, type of the diet or other factors. Some studies have shown decreased levels of antioxidant capacity in diabetes mellitus

(Bashan et al., 2009). Furthermore, a decline in cellular antioxidant defense mechanisms, including the glutathione redox system, vitamin C and E has been reported (Evans et al., 2002).

In conclusion, most of type 2 diabetic patients are presented with insulin resistance, glycaemic control seemed to be independent on insulin resistance in type 2 diabetic patients a glycaemic regulation is independent on oxidative stress in insulin resistance type 2 diabeticpatients.

Table 1. Results of glycosylated hemoglobin (HbA_{1c}), fasting insulin (FI), blood glucose (FBG), triglyceride (Tg), malondialdehyde (MDA) and glutathione-S-transferase (GST) levels in the study groups

Parameter	Group	No	Mean ± SD	Range	P value
HbA_{1c} (%)	Patients Control	93 19	10.44 ± 2.74 5.67 ± 0.99	5.97 - 17.25 3.20 - 6.72	0.001
Insulin (µIU/ml)	Patients Control	93 19	13.40 ± 12.50 5.97 ± 3.09	2.92 - 73.06 3.25 - 17.39	0.001
FBG (mmol/L)	Patients Control	93 19	204.60 ±73.7 102.70±16.50	68.07- 414.64 75.80 - 146.18	0.001
TG (mmol/L)	Patients Control	93 20	6.64 ± 4.87 3.27±1.51	1.29 - 31.41 1.38 - 7.29	0.001
MDA (µM)	Patients Control	93 19	18.60 ± 8.60 24.00 ± 18.40	2.83 - 103.52 7.13 - 34.73	0.06
GST (U/ml)	Patients Control	93 19	12.20 ± 8.11 5.23 ± 8.15	0.00 - 18.75 0.00 - 43.52	0.002

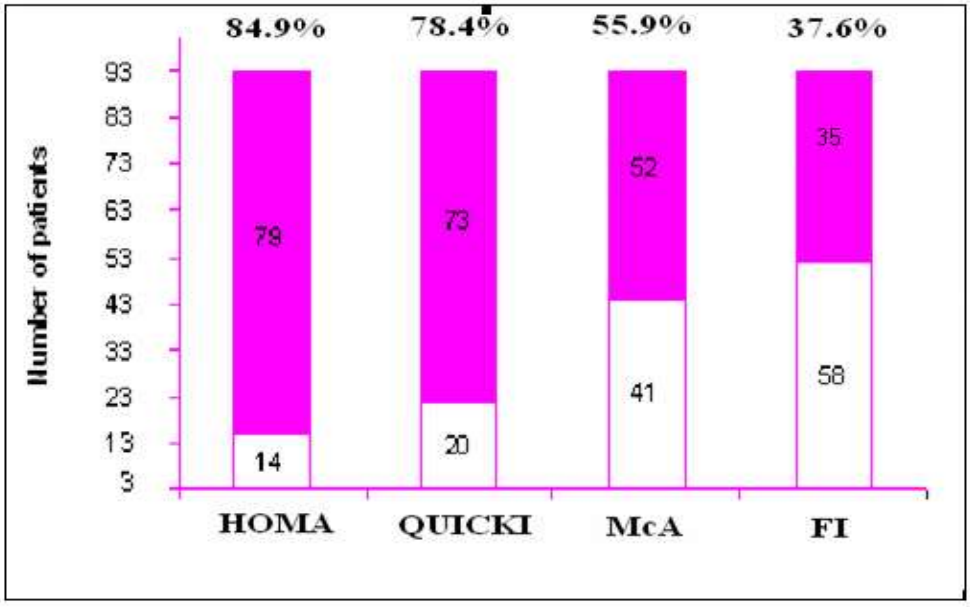

Figure 1. Insulin resistance (IR) among type 2 diabetes mellitus.

The number and percentage of patients with insulin resistance evaluated by HOMA, QUIKI, McA and FI methods, Insulin resistance was found in 79 (84.9%), 63 (73.4%), 52 (55.9%) and 35 (37%) out of the 93 diabetic patients by HOMA, QUICKi, McA and FI methods respectively.

Table 2. The Incidence of insulin resistance and sensitivity in diabetic and the control groups

Index	Insulin resistant subjects		Insulin sensitive subjects	
	Patients	Control	Patient	Control
HOMA	79 (84.9%)	1(5%)	14 (15.05%)	19 (95%)
QUICKI	73 (78.49%)	1(5%)	20 (21.5%)	19 (95%)
MCA	52 (55.91%)	0 (0%)	41 (44.08%)	20 (0%)
FI	35 (37.63%)	1 (5%)	58 (62.36%)	19 (95%)

Table 3. Glycosylated hemoglobin (HbA_{1c}), malodialdehyde (MDA) and glutathione-S-transferase (GST) levels in insulin resistant (IRP), insulin sensitive (ISP) type 2 diabetic patient and the control groups

Parameter	Group	No	Mean ± SD	Range	P value
HbA_{1c} (%)	IRP	79	10.38 ± 2.63	5.97- 15.81	0.001 A
	ISP	14	10.77 ± 3.35	5.93- 17.25	0.001 B
	Control	19	5.80 ± 1.03	3.20- 6.92	NS C
MDA (μM)	IRP	79	23.90 ±19.50	3.90-103.50	NS A
	ISP	14	24.50 ± 11.40	11.0 -39.30	NS B
	Control	19	18.38 ± 8.78	7.10- 26.70	NS C
GST (U/ml)	IRP	79	11.71 ± 7.63	0.00- 43.51	0.005 A
	ISP	14	14.80 ± 10.20	0.00- 39.89	0.005 B
	Control	19	5.21 ± 8.38	0.00-18.75	NS C

A: Insulin resistant patients (IRP) vs control group, B: Insulin sensitive patients (ISP) vs control group, C: Insulin resistant patients (IRP) vs Insulin sensitive patients (ISP).

References

1. Adbul-Ghani M, Jenkinson C, Richardson D, Tripathy D and Defronzo R. Insulin secretion and action in subject with impaired fasting glucose tolerance: results from the Veterans Administration Genetic Epidemiology Study. Diabetes 2006; 55:1430-1435.
2. Ahmed R. The Physiological and Biochemical Effects of Diabetes on The Balance between Oxidative Stress and Antioxidant Defense System. J Isla Academy Scie 2005; 15(1): 31-42.
3. Amato M, Galluzzo A, Merlino S and et al. Lower insulin sensitivity differentiates hirsute from non-hirsute Sicilian women with polycystic ovary syndrome. Eur J End 2006; 155: 859-865.
4. Bablock W. General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988; 26: 783-790.
5. Bashan N and et al., Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen

species. Physio Rev 2009; 89(1): 27-71.
6. Benrebai M, Abidli N, Soad M, Nasr and Benlatreche C. Oxidative stress status in type 2 diabetic patients in eastern Algeria. World Appl Sci J 2008; 4(5): 714-719.
7. Cefalu W. Insulin resistance: cellular and clinical concepts. Exp Bio Med 2001; 226: 13-26.
8. DeFronzo M. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycaemic clamp. Diabet Care 1999; 22: 1462-1470.
9. Defronzo R. From the triumvirate to the ominous octet: anew paradigm for the treatment of the type 2 diabetes mellitus. Diabetes 2009; 58: 773-795.
10. Erus U, Mmed, Rnlo J and et al. Long-Term Predictors of Insulin Resistance Role of lifestyle and metabolic factors in middle-aged men. Diabet Care 2007; 30: 2928-2933.
11. Evans J, Goldfine I, Maddux B and Grodsky G. Oxidative Stress and Stress-Activated Signaling Pathways: A Unifying Hypothesis of Type 2 Diabetes. End Rev 2002; 23: 599-622.
12. Fossati P and Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982; 28 (10): 2077-2080.
13. Goud M, Nayal B, Devi S, Sathisha T, Shivashanker and Devaki R. Relation of calculated HbA_{1C} with fasting plasmaglucose and duration of diabetes. Internat J Appl Biol and Pharmaceut Technol 2011; 2: 58-63.
14. Guidet B and Shah S. Enhanced in vivo H_2O_2 generation by rat kidney in glycerol- induced renal failure. AM J Physio 1989; 1257: 440-444.
15. Habig W, Pabst M and Jakby W. Glutathione-S-transferase. The enzymatic step in mercapturic acid formation. J Biol Chem 1974; 22 (25): 7130-7139.
16. Ikekpeazu E, Neboh E, Ejezie F, Ibegbu M and Ike I. Oxidative Stress and Glycaemic Control in Type 2 Diabetic Patients in Enugu, South-East Nigeria . Ann Med Heal Sci Res Jan 2011; 1(1): 123-128.
17. Jin H and Pan Y. Angiotensin type-1 receptor blockade with losartan increases insulin sensitivity and improves glucose homeostasis in subjects with type 2 diabetes and nephropathy. Nephrol Dial Transplant 2007; 22: 1943-1949.
18. Kahn B and Flier J. Obesity and insulin resistance, J Clin Invest 2000; 106:110-116.
19. Kalaivanam K, Dharmalingam M, Marcus S. Lipid peroxidation in type 2 diabetes mellitus. Int J Diab Dev Ctries 2006; 26: (1): 23-29.
20. Kilpatrick E. Glycosylated haemoglobin in the tear. J Clin Pathol 2000; 53 (5): 335-339.
21. Kojo S. Vitamin C: basic metabolism and its function as an index of oxidative stress. Curr Med Chem 2004; 11: 1041-1064.
22. Lalitha B, Sairam C, Ushakiranmayi G, Sudhakar P and Vijetha. Comparative Study of Oxidative Stress, Clinical Complications and Incidence of Type-II Diabetics in Different Age Groups. Int J Med Res 2010; 1 (1): 59-67.
23. Lebovitz H. Insulin resistance-a common link between type 2 diabetes and cardiovascular disease. Diab Obes Met 2006; 8: 237-249.
24. Liese A, Schulz M, Fang F et al. Dietary glycaemic index and glycaemic load, carbohydrate and fiber intake and measures of insulin sensitivity, secretion and adiposity in the insulin resistance atherosclerosis study. Diabet Care 2005; 28: 2832-2838.
25. Lukshmy M, Shalika P, Sudheera J. Comparison of insulin resistance by indirect methods - HOMA, QUICKI and McAuley- with fasting insulin in

patients with type 2 diabetes. J Heal Aca Sci 2006; 5 (1): 1-8.
26. McAuley K, Williams S, Mann J. Diagnosing insulin resistance in the general population. Diabet Care 2001; 24: 460-464.
27. Mentink C, Kilhovd B, Rondas-Colbers G et al. Time course of specific AGEs during optimized glycaemic control in type 2 diabetes. Nether J Med 2006; 64: 10-16.
28. Mittler R and et al. Reactive oxygen gene network of plants, Trends Plant Sci 2004; 9: 490-498.
29. Moussa A and Romanlan J. Oxidative stress in diabetes mellitus. Biophys 2008; 18 (3): 225-236.
30. Murano G, Barbatelli V, Parisani C, Latini G, Muzzonigro M, Castellucci and Cinti S. Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice. J Lipid Res 2008; 49: 1562-1568.
31. Ralph A. DeFronzo M. Pathogenesis of type 2 diabetes mellitus. Med Clin N Am 2004; 88: 787-835.
32. Rewers M, Zaccaro D, D'Agostino R, Haffner S et al. Insulin sensitivity, insulinemia and coronary artery disease. The insulin atherosclerosis study. Diabet Care 2004; 27: 781-787.
33. Sanocka D and Kurpisz M. Reactive oxygen species and sperm cells. Repro Bio Endo 2004; 2:12-25.
34. Sathiyapriya N, Selvaraj Z, Bobby M. Perturbation of erythrocyte antioxidant barrier, lipid peroxidation and protein carbonylation in non-diabetic first degree relatives of patients with type 2 diabetes. Diabet Res Clin Pract 2007; 78: 171-175.
35. Scott M, James I, Stephen R, Karen A, Robert H, Barry A, David J, Ronald M, Peter J, Sidney C, John A, and Fernando C. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circ 2005; 112: 2735-2752.
36. Seufi A, Ibrahim S, Elmaghraby T and Hafez E. Preventive effect of the flavonoid, quercetin, on hepatic cancer in rats via oxidant/antioxidant activity: molecular and histological evidences. J Exp Clin Cancer Res 2009; 28 (80): 1186-1190.
37. Stanbio Laboratory data. Company research and investing information. analyzers, hemoglobin systems, influenza A and B kits. RSV kits, blood lab test 2011.
38. Velazquez E, Winocour P, Kesteven P, Alberti K and laker M. Relation of lipid peroxides to macrovasclar disease in type 2 diabetes. Diabet Med 1991; 8: 752-758.
39. Wang Y. Free radical and glutathione peroxidase. Pharm J Chin PLA 2005; 21: 369-371.
40. Weisberg S, McCann C, Desai M, Rosenbaum M, Leibel R and Ferrante A. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003; 112: 1796-1808.
41. World Health Organization. Laboratory Diagnosis and Monitoring of Diabetes Mellitus 2002.
42. Young S, Mainous M, and Carnemolla M. Hyperinsulinemia and Cognitive Decline in a Middle-Aged Cohort. Diabet Care 2006; 29: 2688-2693.