Research Article

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The independent relationship between hemoglobin A$_{1c}$ and homeostasis model assessment of insulin resistance in non-diabetic subjects

Diyabetik olmayan bireylerde hemoglobin A$_{1c}$ ve homeostasis model insülin direnci arasındaki bağımsız ilişki

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Abstract

Introduction: Determining the factors affecting hemoglobin A$_{1c}$ (HbA$_{1c}$) levels may help better interpretation of HbA$_{1c}$ values. In this study, we investigated if insulin resistance is a significant parameter contributing to the variability of HbA$_{1c}$ values.

Methods: We retrospectively analyzed serum fasting glucose, fasting insulin, 2 h glucose and HbA$_{1c}$ records of 18–85 years aged outpatients who underwent a 75 g oral glucose tolerance test (OGTT) in our hospital during the period January 2010–May 2014. Homeostasis model assessment of insulin resistance (HOMA-IR) ≥ 2.5 was defined as insulin resistant.

Results: Insulin resistant subjects with normal glucose tolerance had significantly higher HbA$_{1c}$ and fasting glucose levels compared to insulin sensitive subjects with normal glucose tolerance (p = 0.002, p < 0.001, respectively). Similarly, insulin resistant subjects with pre-diabetes had significantly higher HbA$_{1c}$ and 2-h glucose levels compared to insulin sensitive subjects with pre-diabetes (p = 0.016, p = 0.013, respectively). Regression analysis showed that HOMA-IR(log) is associated with HbA$_{1c}$ levels independent of fasting and 2h glucose concentrations (p < 0.001). Age was the variable with highest standardized β coefficient in regression model.

Conclusion: Our data showed that HOMA-IR is associated with glycated hemoglobin values independent of glycemic status and the effect of age on HbA$_{1c}$ values should not be ignored in non-diabetic subjects.

Keywords: HbA$_{1c}$; Insulin resistance; Age; Diabetes mellitus; HOMA-IR.

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Özet

Amaç: Hemoglobin A$_{1c}$ (HbA$_{1c}$) sonuçlarını etkileyen faktörlerin belirlenmesi bu testin daha iyi yorumlanmasını sağlayabilir. Bu çalışmada insülin direncinin HbA$_{1c}$ değerlerini etkileyen anlamlı bir parametre olup olmadığı araştırılmıştır.

Yöntem: Retrospektif olarak hastanemizde Ocak 2010-Mayıs 2014 tarihleri arasında 75 g oral glukoz tolerans testi (OGTT) yapılan 18–85 yaşlarındaki ayaktan hastaların serum açlık glukozu, açlık insülini, 2.saat glukoz ve HbA$_{1c}$ değerlerini analiz ettik. Homeostasis model insülin direnci (HOMA-IR) ≥ 2.5 değeri insülin direnci olarak kabul edildi.

Bulgular: Normal glukoz toleranslı insülin dirençli bireyler normal glukoz toleranslı insülin duyarlı bireylerde göre daha yüksek HbA$_{1c}$ ve açlık glukoz seviyelerine sahiptir (srasıyla p = 0.002, p < 0.001). Benzer şekilde prediabetli insülin dirençli bireyler, prediabetli insülin duyarlı bireylerde göre daha yüksek HbA$_{1c}$ ve 2.saat glukoz seviyelerine sahiptir (srasıyla p = 0.016, p = 0.013). Regresyon analizinde HOMA-IR(log), açlık ve 2.saat glukoz
konsantrasyonlarından bağımsız olarak HbA₁c seviyeleri ile ilişkili bulunmuştur ($p < 0.001$). Yaş regression modelinde en yüksek standardize beta katsayısı sahip değişken olarak bulunmuştur.

**Sonuç:** Verilerimiz glisemik durumdaki bağımsız olarak HOMA-IR’nin glikoz ile hemoglobin düzeyleri ile ilişkili olduğunu ve diabetik olmayan bireylerde yaşın HbA₁c düzeylerine etkisi göz ardı edilmemelidir.

**Anahtar Kelimeler:** HbA₁c; İnsülin direnci; Yaş; Diabetes mellitus; HOMA-IR.

**Introduction**

Insulin resistance (IR) is defined as the impaired ability of target tissues of fat, liver, and muscle to show various metabolic effects of insulin, including glucose uptake [1]. Insulin resistance plays an important pathophysiological role in the development of diabetes, dyslipidemia, hypertension, and cardiovascular disease [2, 3]. Prospective studies have shown that it is a powerful predictor of the likelihood of an individual developing diabetes or cardiovascular disease [4]. Accurate measurement of IR requires complex techniques that are expensive and time-consuming. A number of surrogate indices of IR had been developed. The homeostatic model of assessment-insulin resistance (HOMA-IR), which uses fasting insulin and glucose levels to calculate IR, is the most widely used [5]. However, HOMA-IR results are reasonably correlated with those of clamping studies (the “gold standard”).

Glycated hemoglobin (HbA₁c) is well recognized and widely used as a measure of glycemic control reflecting the mean blood glucose level over the preceding weeks to months. Until recently, hemoglobin A₁c has only been used to monitor people already diagnosed with diabetes, serving as the gold-standard measure of glycemic levels over a 3-month period. More recently, as with measures of glucose, HbA₁c levels have been used to describe a continuum of risk for the development of diabetes and associated conditions. Because it can be measured regardless of food intake, A₁c is simpler than fasting plasma glucose (FPG) or oral glucose tolerance tests [6].

Recently, the International Expert Committee recommended that HbA₁c be added to the diagnosis of diabetes mellitus (DM); the 2010 American Diabetes Association (ADA) clinical practice recommendation defining HbA₁c levels of over 6.5% as DM, and an HbA₁c between 5.7 and 6.4% as pre-diabetes [7, 8]. It was shown that pre-diabetes is associated with insulin resistance which is a risk factor for development of type 2 diabetes. How well a HbA₁c level in the pre-diabetic range (5.7%–6.4%) predicts insulin resistance is not clear. Publications associated with the relationship between HbA₁c and insulin resistance are limited. Therefore, the aim of the current study was to clarify the relationship between insulin resistance and HbA₁c.

**Materials and methods**

We retrospectively analyzed serum fasting glucose, fasting insulin, 2 h glucose and HbA₁c records of 18–85 years-old outpatients who underwent a 75 g OGTT in Department of Biochemistry, Tepecik Teaching and Research Hospital during the period January 2010–May 2014. The samples for all parameters were collected in the same day. Patients with a serum creatinine level above reference range and patients with DM according to ADA criteria (fasting glucose > 125 mg/dl or 2 h glucose > 199 mg/dL or HbA₁c > 6.4%) were excluded. The present study included a total of 365 patients of whom 281 were women and 84 were men. The study was conducted with the approval of the Local Hospital’s Ethics Committee.

Glucose tolerance status was assessed with the 75-g OGTT and serum fasting glucose defined according to the 2010 ADA criteria [8]. Our study used the HOMA-IR as the diagnostic criteria for insulin resistance and HOMA-IR ≥ 2.5 was identified as an indicator of insulin resistance. HOMA-IR was calculated using the following formula: HOMA-IR = fasting serum glucose (mg/dL) × fasting serum insulin value (μU/mL)/405 [5]. Serum glucose levels were measured by the hexokinase method using the Olympus AU 2700 analyzer (Olympus Diagnostics. GmbH, Hamburg, Germany).

Serum insulin levels were measured using direct chemiluminescence technology on Siemens Immulite 2000 XPI analyzer (Siemens Healthcare Diagnostics, Deerfield, IL, USA). HbA₁c analysis was performed by affinity chromatography HPLC on Primus Ultra2 Analyzer (Primus Corporation, Kansas City, Kansas, USA).

**Statistical methods**

Statistical analyses were conducted using the statistical package SPSS version 17 (SPSS Inc., Chicago, IL, USA). $p$-Value $< 0.05$ was considered as statistically significant. Data were expressed as mean ± SD or with 95% confidence intervals (CI). Independent samples t-test and $\chi^2$ test were used for comparison of insulin resistant and insulin
sensitive groups. Multivariate linear regression analysis was used for determining the association between HbA1c and independent variables. Variables with non-normal distribution were log transformed.

**Results**

A total of 365 non-diabetic subjects were included in the study. Mean age of the subjects were 40 ± 11 and 281 (77%) of the subjects were female, while 84 (23%) of the subjects were male. There were 191 individuals with normal glucose tolerance and 174 individuals with pre-diabetes (impaired fasting glucose, impaired glucose tolerance, or both according to ADA criteria). Insulin resistance (HOMA-IR > 2.5) were detected in 147 (40%) of the subjects. Table 1 shows the characteristics of the study population.

The subjects were divided into two groups according to their HOMA-IR values: group 1, insulin sensitive (HOMA-IR ≤ 2.5) and group 2, insulin resistant (HOMA-IR > 2.5). HbA1c and other biochemical parameters of insulin resistant and insulin sensitive subjects were compared. Analysis showed that insulin resistant subjects with normal glucose tolerance had significantly higher HbA1c and fasting glucose levels compared to insulin sensitive subjects with normal glucose tolerance (p = 0.002, p < 0.001, respectively). Similarly, insulin resistant subjects with pre-diabetes had significantly higher HbA1c and 2-h glucose levels compared to insulin sensitive subjects with pre-diabetes (p = 0.016, p = 0.013, respectively) (Table 2).

Table 1: Characteristics of the study population.

| n = 365 | Age (mean ± SD) 40 ± 11 |
|---------|-------------------------|
| Sex     | Male 84 (23)  Female 281 (77) |
| OGTT    | Normal glucose tolerance 191 (52)  Pre-diabetes 174 (48) |
| Pre-diabetes | IFG 112 (64)  IGT 11 (6)  IFG and IGT 51 (30) |
| HOMA-IR | > 2.5 147 (40)  ≤ 2.5 218 (60)  HbA1c 5.5 ± 0.4 |

Data are expressed as mean ± SD or n (%). IFG, Impaired fasting glucose; IGT, impaired glucose tolerance.

To estimate the independent relationship between HbA1c and HOMA-IR, multiple linear regression analysis was used. Age, gender, fasting glucose, 2-h glucose and HOMA-IR were entered in the regression model as independent variables. Since HOMA-IR values had skewed distribution, log transformed HOMA-IR was used in analysis. Regression analysis showed that age, fasting glucose, 2-h glucose, and log transformed HOMA-IR values are significant predictors of HbA1c. Higher HOMA-IR values are associated with higher HbA1c levels in non-diabetic subjects. Standardized β coefficients demonstrate the relative importance of predictor variables in regression model. In the regression model age showed the highest standardized β coefficient. In non-diabetic subjects age was the most important factor affecting HbA1c values (Table 3). HbA1c values were also presented in different age groups (Table 4).

Dividing the study population as normal and pre-diabetic subjects and re-performing the regression analysis did not change the results fundamentally. Age was still most effective factor in predicting HbA1c values in both normal group and pre-diabetic group. Log HOMA was still a significant variable (data not shown).

To visualize the data a regression plot showing the relation between HbA1c and HOMA-IR was generated. The relation between HbA1c and HOMA-IR was similar in different sample groups (group 1: fasting glucose < 96 mg/dL, group 2: fasting glucose > 96 mg/dL) (Figure 1).

**Discussion**

It was clearly reported that lower HbA1c values were associated with reduced microvascular and macrovascular complications in diabetic patients [9]. In type 2 diabetes a HbA1c target level of < 7% is currently recommended by ADA and HbA1c is the basis guiding diabetes therapy. Recently ADA recommended using HbA1c also for the diabetes diagnosis and consolidated its importance in diabetes mellitus. The use of HbA1c as a diagnostic criterion necessitates a more accurate measurement and careful interpretation. Several factors other than plasma glucose levels were reported to affect glycated hemoglobin levels. Any condition affecting turnover of red blood cells like hemolytic anemia and iron deficiency affect HbA1c levels. Previous studies showed that genetic-ethnic factors, sex hormones and age are associated with HbA1c values [10–14]. In the present study we report that HOMA-IR is a factor affecting HbA1c values independent of fasting and 2-h post-load glucose concentrations. The relationship between glycated hemoglobin...
levels and glucose levels are higher in diabetic patients than in non-diabetic patients therefore it can be concluded that non-glycemic factors affecting HbA1c levels are more important in non-diabetic patients [15].

Previous studies investigated the relationship between insulin resistance and HbA1c. Gallwitz et al. showed that with increasing HbA1c levels, there was a statistically significant increase in HOMA-IR in patients with type 2 DM [16]. Heianza et al. reported that in subjects without a history of diabetes a HbA1c level of > 5.9% were significantly associated with higher HOMA-IR values [17]. In another study Borai et al. showed that the correlation between HbA1c and insulin resistance were higher in subjects with normal glucose tolerance than in patients with pre-diabetes and diabetes [18]. None of the aforementioned studies reported corrected results independent of glycemic status. Venkataraman et al. showed that in a multivariate regression model with HbA1c as dependent variable HOMA-IR(log) were significantly associated with HbA1c independent of fasting glucose but the effect of post-load glucose on HbA1c was lacking [19]. One recent study indicated that HbA1c was associated with HOMA-IR independent of 0- and 120-min glucose in pregnant women with gestational diabetes mellitus [20]. The results of our

### Table 2: Demographic and biochemical parameters of insulin resistant and insulin sensitive subjects.

| Variables                  | Normal glucose tolerance | Pre-diabetes |
|----------------------------|--------------------------|--------------|
| Age                        | HOMA ≥ 2.5 (n=60)        | HOMA < 2.5 (n=131) | p-Value  |
| Female/male                | 37 ± 11                  | 37 ± 10      | 0.923     |
| HbA1c (%)                  | 73%                      | 82%          | 0.147*    |
| 2-h glucose (mg/dL)        | 5.5 ± 0.4                | 5.3 ± 0.4    | 0.002     |
| Fasting glucose (mg/dL)    | 96 ± 22                  | 91 ± 22      | 0.229     |
| HOMA-IR (log)              | 0.010                    | 0.279        | < 0.001   |
| p-Value                    | 0.279                    | 0.192        | < 0.001   |
| %95 CI for B               | 0.007–0.014              | 0.133–0.392  |

### Table 3: Multivariate linear regression model with HbA1c as dependent variable.

| Variables                  | Regression coefficient B | Standardized coefficient β | p-Value | %95 CI for B |
|----------------------------|--------------------------|-----------------------------|---------|-------------|
| Age                        | 0.010                    | 0.279                       | < 0.001 | 0.007–0.014 |
| HOMA-IR (log)              | 0.263                    | 0.192                       | < 0.001 | 0.133–0.392 |
| Fasting glucose (mg/dL)    | 0.008                    | 0.218                       | < 0.001 | 0.004–0.011 |
| 2-h glucose (mg/dL)        | 0.001                    | 0.100                       | 0.040   | 0.0005–0.0025 |
| Sex                        | 0.056                    | 0.056                       | 0.210   | –0.032–0.145 |

### Table 4: HbA1c values in different age groups.

| Age          | HbA1c normalization (n=191) | HbA1c pre-diabetes (n=174) |
|--------------|-----------------------------|---------------------------|
| 18–30 (n=75) | 5.31 ± 0.38                 | 5.57 ± 0.31               |
| 31–40 (n=107)| 5.38 ± 0.38                 | 5.64 ± 0.39               |
| 41–50 (n=107)| 5.55 ± 0.44                 | 5.74 ± 0.40               |
| 51–60 (n=61) | 5.66 ± 0.34                 | 5.87 ± 0.31               |
| > 60 (n=15)  | 5.90 ± 0.45                 | 6.00 ± 0.26               |

### Figure 1: Scatterplot showing correlation between HOMA-IR and HbA1c in different sample groups. Group 1: fasting glucose < 96 mg/dL. Group 2: fasting glucose > 96 mg/dL.
study reveal that HbA1c is associated with HOMA-IR independent of fasting and post-load glucose status also in non-diabetic subjects.

Glycation is the nonenzymatic attachment of free aldehyde groups of carbohydrates to the unprotonated free amino groups of proteins [21]. The binding of glucose molecules to potential glycation sites in hemoglobin molecule leads to formation of HbA1c. Via condensation with glucose, hemoglobin A first forms a labile intermediate adduct, which is thereafter rearranged to the more stable ketoamine adduct (HbA1c) form [22]. Physiological factors like pH, inorganic phosphate, oxidative stress, deglycation, and Schiff base inhibitors can affect the rate of HbA1c formation [23–27]. Another point to be considered is that glycation of hemoglobin occurs in the intracellular compartment. Previously, it was demonstrated that the erythrocyte glucose–to–plasma glucose concentration ratio may affect hemoglobin glycation and contributes to the variation in HbA1c levels [28].

Oxidative stress is a process that was proposed to be associated with the multifactorial etiology of insulin resistance. It was shown that plasma markers of oxidative stress were correlated with the degree of insulin resistance [29, 30]. Oxidative stress which is a factor co-existing with insulin resistance may also be responsible for the increased hemoglobin glycation. Oxidative stress biomarkers as lipid peroxides were reported to be associated with hemoglobin glycation [31, 32]. LDL oxidation was also suggested to increase HbA1c values [33]. Furthermore there is evidence that antioxidants can partially inhibit the formation of HbA1c [31].

Consistent with previous studies, age was a significant factor affecting HbA1c levels independent of glycemia [13, 34]. However the mechanisms involved in the age related HbA1c increase remain to be established. Since our model showed that age was the most effective factor contributing to the variation of HbA1c levels in non-diabetic subjects it should not be ignored when interpreting an HbA1c result. The question whether age-specific diagnostic and treatment criteria would be appropriate was previously mentioned [13].

Due to its retrospective design the current study has some limitations. Firstly the information of some possible confounding variables (e.g. BMI) could not be gathered; therefore confounding factors may exist. Second, HOMA-IR is not the gold standard method for measuring insulin sensitivity. The euglycemic hyperinsulinemic clamp technique is the gold standard for quantifying insulin sensitivity; however, this technique requires insulin infusion and repeated blood sampling. HOMA-IR is a relatively simple method to determine insulin sensitivity. It can be calculated from a single blood sample and it was reported to have a linear correlation with glucose clamp technique [35].

In conclusion, our data showed that HOMA-IR is associated with glycated hemoglobin values independent of glycaemia and age is a very important factor affecting HbA1c values in non-diabetic subjects.

Conflict of interest statement: All authors declare that there is no conflict of interest regarding the publication of this article.

References

1. Reaven GM. Pathophysiology of insulin resistance in human disease. Physiol Rev 1995;75:473–86.
2. DeFronzo RA, Ferrannini E. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991;14:173–94.
3. Eckel RH, Grundy SM, Zimet PZ. The metabolic syndrome. Lancet 2005;365:1415–28.
4. Lillioja S, Mott DM, Howard BV, Bennett PH, Yki-Jarvinen H, Freymond D, et al. Impaired glucose tolerance as a disorder of insulin action. Longitudinal and cross-sectional studies in Pima Indians. N Engl J Med 1988;318:1217–25.
5. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
6. Saudek CD, Herman WH, Sacks DB, Bergenson RM, Edelman D, Davidson MB. A new look at screening and diagnosing diabetes mellitus. J Clin Endocrinol Metab 2008;93:2447–53.
7. International Expert Committee. International Expert Committee report on the role of the A1c assay in the diagnosis of diabetes. Diabetes Care 2009;32:1277–34.
8. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010;33:62–9.
9. Stratton IM, Adler AI, Neil HA, Matthews DR, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 2000;321:405–12.
10. Herman WH, Ma Y, Uwaifo G, Haffner S, Kahn SE, Horton ES, et al. Diabetes prevention program research group. Differences in A1c by race and ethnicity among patients with impaired glucose tolerance in the diabetes prevention program. Diabetes Care 2007;30:2453–7.
11. Cohen RM, Snieder H, Lindsell CJ, Bevan H, Hawa M, Blinko S, et al. Evidence for independent heritability of the glycation gap (glycosylation gap) fraction of HbA1c in nondiabetic twins. Diabetes Care 2006;29:1739–43.
12. Snieder H, Sawtell PA, Ross L, Walker J, Spector TD, Leslie RD. HbA1c levels are genetically determined even in type 1 diabetes: evidence from healthy and diabetic twins. Diabetes 2001;50:2858–63.
13. Pani LN, Korenda L, Meigs JB, Driver C, Chamany S, Fox CS, et al. Effect of aging on A1c levels in individuals without diabetes: evidence from the Framingham Offspring Study and the National Health and Nutrition Examination Survey 2001–2004. Diabetes Care 2008; 31:1991–6.

14. Page-Wilson G, Goulart AC, Rexrode KM. Interrelation between sex hormones and plasma sex hormone-binding globulin and Hemoglobin A1c in healthy postmenopausal women. Metab Syndr Relat Disord 2009;7:249–54.

15. van’t Riet E, Alssema M, Rijkelijkhuizen JM, Kostense PJ, Nijpels G, Dekker JM. Relationship between A1C and glucose levels in the general Dutch population: the new Hoorn study. Diabetes Care 2010;33:61–6.

16. Gallwitz B, Kazda C, Kraus P, Nicolay C, Schernthaner G. Contribution of insulin deficiency and insulin resistance to the development of type 2 diabetes: nature of early stage diabetes. Acta Diabetol 2013;50:39–45.

17. Heianza Y, Arase Y, Fujihara K, Tsuji H, Saito K, Hsieh SD, et al. High normal HbA1c levels were associated with impaired insulin secretion without escalating insulin resistance in Japanese individuals: the Toranomon Hospital Health Management Center Study 8 (TOPICS 8). Diabet Med 2012;29:1285–90.

18. Borai A, Livingstone C, Abdelaal F, Bawazeer A, Keti V, Ferns G. The relationship between glycosylated haemoglobin (HbA1c) and measures of insulin resistance across a range of glucose tolerance. Scand J Clin Lab Invest 2011;71:168–72.

19. Venkataraman K, Kao SL, Thai AC, Salim A, Lee JJ, Heng D, et al. Ethnicity modifies the relation between fasting plasma glucose and HbA1c in Indians, Malays and Chinese. Diabet Med 2012;29:911–7.

20. Pan J, Zhang F, Zhang L, Bao Y, Tao M, Jia W. Influence of insulin sensitivity and secretion on glycosylated albumin and hemoglobin A1c in pregnant women with gestational diabetes mellitus. Int J Gynaecol Obstet 2013;121:252–6.

21. Makris K, Spanou L. Is there a relationship between mean blood glucose and glycated hemoglobin? J Diabetes Sci Technol 2011;5:1572–83.

22. Mortensen HB. Glycated hemoglobin. Reaction and biokinetic studies. Clinical application of hemoglobin A1c in the assessment of metabolic control in children with diabetes mellitus. Dan Med Bull 1985;32:309–28.

23. Cohen RM, Franco RS, Khera PK, Smith EP, Lindsell CJ, Ciraolo PJ, et al. Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c. Blood 2008;112:4284–91.

24. Gould BJ, Davie SJ, Yudkin JS. Investigation of the mechanism underlying the variability of glycated haemoglobin in non-diabetic subjects not related to glycaemia. Clin Chim Acta 1997;260:49–64.

25. Kunika K, Itakura M, Yamashita K. Inorganic phosphate accelerates hemoglobin A1c synthesis. Life Sci. 1989;45:623–30.

26. Delpierre G, Collard F, Fortpied J, Van Schaftingen E. Fructosamine 3-kinase is involved in an intracellular deglycation pathway in human erythrocytes. Biochem J 2002;365:801–8.

27. Szwergold BS, Howell SK, Beisswenger PJ. Transglycation—a potential new mechanism for deglycation of Schiff’s bases. Ann N Y Acad Sci 2005;1043:845–64.

28. Khera PK, Joiner CH, Carruthers A, Lindsell CJ, Smith EP, Franco RS, et al. Evidence for interindividual heterogeneity in the glucose gradient across the human red blood cell membrane and its relationship to hemoglobin glycation. Diabetes 2008;57:2445–52.

29. Henriksen EJ, Diamond-Stanic MK, Marchionne EM. Oxidative stress and the etiology of insulin Resistance and type 2 diabetes. Free Radic Biol Med 2011;51:993–9.

30. Nourooz-Zadeh J, Rahimi A, Tajaddini-Sarmadi J, Tritzschler H, Rosen P, Halliwell B, et al. Relationships between plasma measures of oxidative stress and metabolic control in NIDDM. Diabetologia 1997;40:647–53.

31. Selvaraj N, Bobby Z, Sathiyaipriya V. Effect of lipid peroxides and antioxidants on glycation of hemoglobin: an in vitro study on human erythrocytes. Clin Chim Acta 2006;366:190–5.

32. Sathiyaipriya V, Bobby Z, Vinod Kumar S, Selvaraj N, Parthibane V, Gupta S. Evidence for the role of lipid peroxides on glycation of hemoglobin and plasma proteins in non-diabetic asthma patients. Clin Chim Acta 2006;366:299–303.

33. Hussein OA, Gefen Y, Zidan JM, Karochero EY, Luder AS, Assy NN, et al. LDL oxidation is associated with increased blood hemoglobin A1c levels in diabetic patients. Clin Chim Acta 2007;377:114–8.

34. Ravikumar P, Bhansali A, Walia R, Shamugasundar G, Ravikiran M. Alterations in HbA1c with advancing age in subjects with normal glucose tolerance: Chandigarh Urban Diabetes Study (CUDS). Diabet Med 2011;28:590–4.

35. Singh B, Saxena A. Surrogate markers of insulin resistance: a review. World J Diabetes 2010;1:36–47.