# EVALUATION AND ASSOCIATION OF Hb-AGE WITH DYSLIPIDEMIA AND BLOOD VISCOSITY IN TYPE 2 DIABETIC ASIAN INDIANS

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**Abstract: The aim of this study was to examine the status of hemoglobin advanced glycated end product (Hb-AGE) in Asian Indians with Type 2 diabetes mellitus with or without dyslipidemia and see if these parameters are close correlated to whole blood viscosity. The subjects were recruited into groups I, II and III which are Type 2 diabetes mellitus with dyslipidemia, normolipidemic Type 2 diabetic patients and non-diabetic healthy controls, respectively. Hb-AGE levels and whole blood viscosity in Groups I and II were significantly higher ($p<0.01$ and $p<0.05$) compared to Group III. However, whole blood viscosity or Hb-AGE between Group I and II were not significantly different. A positive correlation ($r=0.546$) was observed between whole blood viscosity and Hb-AGE, irrespective of dyslipidemia. Increased whole blood viscosity could be due to structural and functional abnormalities of erythrocytes mediated by accumulation of elevated levels of hemoglobin advanced glycation end product. Hb-AGE is found to be correlated to whole blood viscosity but not to dyslipidemia. In this subject population, dyslipidemia and high Hb-AGE may contribute independently to hemorheological aberrations, which may be important factor for development of angiopathy.**

## Introduction

Dyslipidemia is commonly associated with Type 2 diabetes mellitus [1], and is characterized by hypertriglyceridemia and decreased levels of high-density lipoprotein cholesterol [2]. This pattern of dyslipidemia is present in approximately one third of Type 2 diabetic Asian Indians [3].

Prolonged hyperglycemia and dyslipidemia in diabetes affects multiple organ systems and may cause significant morbidity and mortality due to microvascular and macrovascular complications. Elevated plasma and whole blood viscosity (WBV) has been reported in patients with hypercholesterolemia and hypertriglyceridemia, respectively [4,5]. Hemorheological abnormalities may also occur in absence of dyslipidemia [6]. Physicochemical modifications of proteins are often associated with diabetes irrespective of dyslipidemia. Elevated levels of early glycated product and advanced glycation end product (AGE) of erythrocyte membrane proteins and hemoglobin are caused by structural and chemical modifications of these proteins due to hyperglycemia. The alterations in the physicochemical properties of red blood cell proteins may lead to hemorheological abnormalities [7,8].

The development of AGE resulting from non-enzymatic glycation and glycoxidation of proteins is hypothesized to play an important role in the pathogenesis of vascular disease in diabetes [9,10]. Epidemiological data indicates that the degree and duration of hyperglycemia is associated with the micro-vascular and macro-vascular complications of disease. Elevated levels of serum AGE has been reported to correlate with micro-vascular complications such as nephropathy and retinopathy [11]. Levels of advanced glycation end products could be valuable in assessing the risk of progression to diabetic complications. In diabetic patients high AGEs levels are deposited in protein compounds like collagen for a longer time due to their slow turn over rate [12]. AGE formation on the extra cellular matrix results in decreased elasticity of blood vessels [13]. The role of AGE in hemorheology has been poorly researched. Finally, the association of Hb-AGE and dyslipidemia in Type 2 diabetes mellitus has not been established.

Asian Indians are particularly predisposed to develop Type 2 diabetes mellitus and coronary heart disease. Further, macrovascular disease is an important cause of mortality in patients with Type 2 diabetes mellitus. The role of Hb-AGE in the pathogenesis of vascular complications needs more research, in Asian Indians. In order to ensure prevention of rising cardiovascular mortalities among this population, it is necessary to look into all facets of the problem. Keeping this aim in mind, we hypothesized that Hb-AGE is significantly correlated to whole blood viscosity in presence or absence of dyslipidemia , thus contributing to vascular problems in Asian Indians with Type 2 diabetes mellitus.


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## Material and Methods

Type 2 diabetes mellitus patients (n=60, mean age; 44 ± 14 years, range 30 - 70 years) belonging to both genders (males 24, females 45) were selected from outpatient clinics of Department of Internal Medicine, All India Institute of Medical Sciences . The patients were on various combinations of anti-hyperglycemic treatment, and free from severe end-organ damage and acute systemic illnesses. Twenty five normoglycemic, normolipidemic healthy subjects were recruited as controls through local advertisement after excluding diabetes mellitus with standard oral glucose tolerance test [14]. Approval for these studies was taken from the Institute Ethics Committee and informed consent was obtained from all the subjects. They were classified into three groups namely

Group- I: Type 2 diabetes mellitus patients with dyslipidemia (n=30)

Group-II: Normolipidemic Type 2 diabetes mellitus patients (n=30)

Group-III: Non-diabetic normolipidemic controls (n=25)

Type 2 diabetes mellitus was diagnosed based on standard diagnostic criteria [15]. Dyslipidemia was defined as any abnormality of HDL-C, total cholesterol, serum triglycerides and LDL-C based on literature [16].

Blood samples were drawn after 12 hour overnight fast. Blood glucose, total cholesterol, triglycerides, and HDL-C were measured enzymatically using commercial kits (Randox Laboratory, San Francisco, CA, USA) on a semi-automated analyzer (Micro Semi-Autoanalyzer 2000, CL. Micromed Italy). Value of LDL-C was calculated according to Friedewald's equation [17]. $HbA_{1c}$ was estimated using commercial kits (Sigma Co. St.Louis, USA).

Hb-AGE was measured by using its autofluorescent property, according to methodology previously described by us [18]. Briefly, one ml of anti-coagulated blood samples were hemolysed using distilled water. The hemolysate was centrifuged and supernatant was taken and diluted up to 1 mg/ml protein concentration for fluorescence measurement. The fluorescence intensity was measured at excitation and emission wavelength 308 nm and 345 nm respectively, using spectrofluorimeter ( LS 50B Perkin Elmer, Cambridge,UK ).

Two ml of whole blood anticoagulated with EDTA was refrigerated at 40ºC till analyzed. The measurement was done at room temperature using Cone and Plate Rheometer (Rheostress1, Thermo Haake, Germany). The cone or sensor is specially designed for small (~ 1ml) volume samples. The sensor system of the Rheometer consists of a rotating cone and a stationary plate made of Titan alloy (diameter 60mm), on which the former rotates with a fixed speed. The gap setting between the cone and the plate is 0.026 mm, which was calibrated for each measurement. One ml of anticoagulated blood was loaded on the plate for measurement of whole blood viscosity at controlled rate mode under steady shear rate varying between 0 and 150 $s^{-1}$. The excess amount of sample was wiped away with smooth textured tissue paper. The measured values were analyzed using Windows based operating software Rheowin data manager (Thermo Haake; Germany). The apparent whole blood viscosity (WBV) was estimated by taking the mean value of measured WBV in the range of shear rate 40 to 70 $s^{-1}$ . The measured viscosity was standardized at hematocrit 45% using a regression equation [19].

Results were expressed as mean ± SD. All data were examined for normality and variance of homogeneity before statistical analysis. A p value $\leq$ 0.05 was considered significant. Student t test was applied for the comparison of inter-group parameters. The correlations were analyzed using Spearman's correlation coefficient. All statistical analyses were performed using the statistical software SPSS / Windows (SPSS V.10, Chicago, IL).

## Results

The lipid profiles such as total cholesterol (mg/dL), triglycerides (mg/dL), HDL-C (mg/dL), LDL-C (mg/dL) and whole blood viscosity were analyzed and compared with diabetic indicators in all three groups (Table 1).

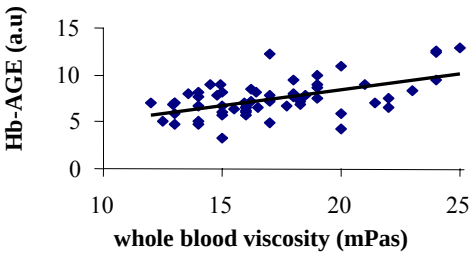


Figure 1: The scatter diagram

The value of whole blood viscosity in Groups I and II was significantly different in comparison with those of group III but did not show any significant difference within these groups. The scatter diagram (Fig. 1) shows significant correlation (r= 0.546, p<0.01) between whole blood viscosity and Hb-AGE in all diabetic patients, while Figure 2 demonstrates a positive relationship (r = 0.33 ) between WBV and $HbA_{1c}$.


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Table 1: List of parameters observed for Groups I, II, III

| Parameters | Group-I (n=30) a | Group-II (n=30) b | Group-III (n= 25) c | P |
|---|---|---|---|---|
| Plasma glucose (mg/dL) | 150.9 ± 44.59 | 167.5 ± 47.73 | 105.7 ± 9.5 | b,c <0.01 |
| Hb-A1c (%) | 8.15 ± 0.52 | 8.07 ± 0.63 | 5.97 ± 0.63 | b,c <0.01 |
| Hb-AGE (a.u) | 7.48 ± 2.66 | 7.46 ± 0.98 | 1.69 ± 0.24 | b,c <0.01 |
| Total Cholesterol (mg/dL) | 192.2 ± 45 | 167.16 ± 24.0 | 168.12 ± 17.97 | a,b < 0.01 |
| Triglycerides (mg/dL) | 214.6 ± 100.9 | 141.2 ± 21.70 | 140.4 ± 24.7 | a,b < 0.01 |
| HDL-cholesterol (mg/dL) | 39.84 ± 2.68 | 41.63 ± 2.04 | 41.32 ± 2.4 | a,b< 0.05 |
| LDL-cholesterol (mg/dL) | 109.48 ± 46.7 | 97.3 ± 23.88 | 97.05 ± 16.6 | Not significant |
| Blood Viscosity (mPas) | 17.04 ± 3.45 | 16.42 ± 2.97 | 8.89 ± 1.68 | b,c <0.05 |

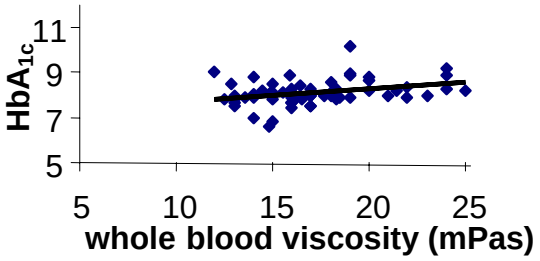


Figure 2: Relationship between WBV and $HbA_{1c}$

In group II the lipid variables, barring HDL-C, were significantly different ($p<0.01$) compared to group I. In group III subjects the pattern of lipid variables were similar to those of group II. Diabetic indicators such as plasma glucose level (mg/dL), $HbA_{1c}$ (%) and Hb-AGE (arbitrary units) levels in Group I and II patients were significantly higher ($p<0.01$) in comparison with Group III (non-diabetic, normolipidemic healthy controls). However these parameters were not significantly different between Groups I and II (Fig. 3).

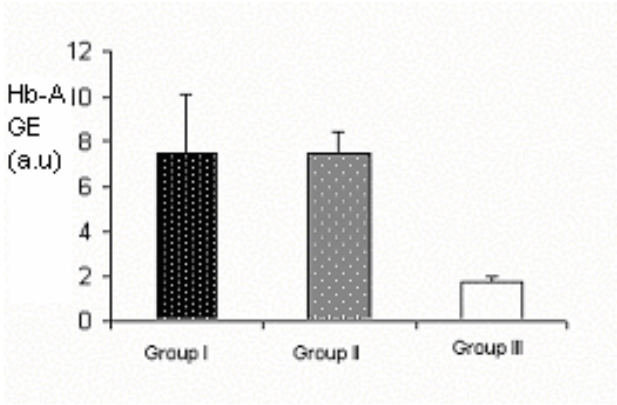


Figure 3: Hb-AGE comparison of Groups I, II, III

**Discussion**

The main objective of this study was to determine the role of Hb-AGE on hemorheology of patients with Type 2 Diabetes mellitus with and without presence of dyslipidema. Hb-AGE has been advocated as an index for long-term monitoring of hyperglycemia because circulating levels of Hb-AGE can almost double in diabetic patients compared to non-diabetic healthy controls [20]. $HbA_{1c}$ and Hb-AGE are the resultant early and advanced products of hyperglycemia and the changes in level of $HbA_{1c}$ may occur simultaneously with Hb-AGE formation as demonstrated by their correlation [18]. Further it is reported that Hb-AGE

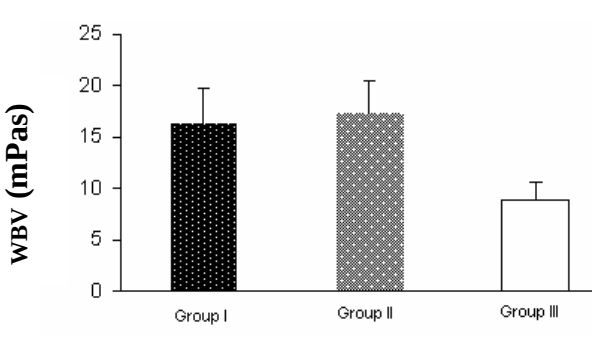


Figure 4: WBV comparison for Groups I, II, III

level declines slower than $HbA_{1c}$ [21].The role of AGE in hemorheology is not clearly defined. Although blood viscosity and its correlation with diabetes has been the subject of investigation for a long time, it has remained a controversial issue. In the present study, a significant increase ($p<0.05$) in apparent WBV was observed in Type 2 diabetic patients compared with healthy normal controls ( Fig.4 ) and the result was consistent with an earlier study which also reported enhanced viscosity [22]. Based on this observation we conclude that hemoglobin glycated end products may affect erythrocyte hemorheological properties as a result of structural and functional modification of hemoglobin molecule, which in turn has a direct bearing on blood flow .


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Some studies have demonstrated that AGEs of different plasma proteins have no influence on plasma viscosity [23] and that a mere increase in $HbA_{1c}$ does not affect WBV [24], but one major lacuna in the latter study was that erythrocytes were exposed to hyperglycemic condition only for a limited time (96 hrs) whereas in diabetic patients erythrocytes are exposed to hyperglycemia for several months. On the other hand there is evidence that a positive correlation existed between whole blood viscosity and $HbA_{1c}$ [25]. Our study, corroborates a positive correlation between $HbA_{1c}$ and WBV in Type 2 diabetic patients ( Fig. 2). It further demonstrates that Hb-AGE, the final adduct, is better correlated with WBV than $HbA_{1c}$ even though the r value is not that high. These results indicate that hemoglobin modified glycated products may have potential role in the hemorheological changes observed in diabetes.

Hyperglycemia can also lead to glycation of erythrocyte membrane proteins [26]. In another study [27] the amount of AGEs was quantified in diabetic erythrocyte peripheral-membrane proteins by an ELISA and a significant correlation established between levels of AGE and $HbA_{1c}$ . The alterations in the glycoprotein membrane of red blood cells as a result of glycation in diabetes, may impair the deformability of red cells and thus contribute to the development of late diabetic sequelae [13]. These issues have not been explored in our study.

An attempt was also made to delineate the inter-relationships between lipid profile, Hb-AGE and whole blood viscosity. We observed increased levels of triglycerides and decreased levels of HDL-C in Asian Indians with dyslipidemia in general, which are consistent with earlier studies in other demographic groups [28]. Understandably, parameters such as LDL cholesterol and total cholesterol were found to be significantly different ($p<0.01$) between groups I and II. Glycosylation of lipoproteins has been examined in children with Type 1 diabetes mellitus [29]. They did not find any significant correlation between glycosylated lipoproteins, glycemia or $HbA_{1c}$ although these patients did exhibit qualitative lipid abnormalities. In another study conducted on an Asian population, it was observed that the proportion of hyperlipidemia was higher in Type 2 diabetes patients with fatty liver than without fatty liver [30]. Some studies have reported that abnormal lipid levels like increased serum triglycerides and low HDL-C are associated with increase in blood viscosity [19,31]. However these studies were done in dyslipidemic patients who were not diabetic. There are other reports suggesting increased blood viscosity in diabetic patients [32] but a correlation between the three factors *viz.* lipid levels, hyperglycemia and blood viscosity has not been looked into. The present study examined this aspect and no consistent inter-relationship pattern emerged between whole blood viscosity, lipid profile and Hb-AGE values in Type 2 diabetic Asian Indians with or without dyslipidemia.

## Conclusion

The observed hemorheological changes could be due to structural and functional abnormalities of erythrocytes mediated by elevated levels of both early and advanced glycated products ($HbA_{1c}$ and Hb-AGE), in Asian Indian patients with Type 2 diabetes mellitus regardless of dyslipidemia. Accumulation of Hb-AGEs and general AGEs together with dyslipidemia may accelerate hemorheological abnormalities associated with chronic micro- and macro-vascular disease in diabetes. These observations may have a potentially important role in diabetic angiopathy and other cardiac complications in patients with accompanying diabetes and lipid disorders. Finally, these observations are significant for Asian Indians who have high prevalence of insulin resistance syndrome, which encompasses both hyperglycemia and dyslipidemia [33].

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## References

[1] FAGOT-CAMPAGNA A., ROLKA D.B., BECKES A., GREGG E.W., NARAYAN K.M. (2000): 'Prevalance of lipid abnormalities, awareness, and treatment in US adults with diabetes', Diabetes., 49, pp. 318

[2] KWITEROVICH P.O. (2002): 'Clinical relevance of the biochemical, metabolic and genetic factors that influence low density lipoprotein heterogeneity', Am. J. Cardiol., 90 (Suppl), pp. 30i - 47i.

[3] MISRA A., LUTHRA K., VIKRAM N.K. (2004): 'Dyslipidemia in Asian Indians: determinants and significance', J Assoc Physicians India., 52, pp. 137 - 142

[4] LEONHARDT H., ARNTZ H.R., KLEMENS U.H. (1977): 'Studies of plasma viscosity in primary hyperlipoproteinaemia', Atherosclerosis, 28(1), pp. 29 - 40

[5] ROBERT S., ROSENSON R.S., SHOTT S., TANGNEY C.C. (2002): 'Hypertriglyceridemia is associated with an elevated blood viscosity', Atherosclerosis., 161, pp. 433 - 439

[6] COPPOLA L., VERRAZO G., LA MARCA C., ZICCARDI P., GRASSIA A., TIRELLI A. (1997): 'Effect of insulin on blood rheology in non-diabetic subjects and in patients with Type 2 diabetes mellitus', Diabetic Med., 14, pp. 959 - 963


ISSN: 1727-1983 © 2005 IFMBE

[7] WATALA C. (1992): 'Hyperglycemia alters the physicochemical properties of proteins in erythrocyte membranes of diabetic patients', Int J Biochem., 24 (11), pp. 1755- 1761

[8] WATALA C., GOLANSKI J., WITAS H., GORBIEL R., GWOZEZINSKI K., TROJENOSKI Z. (1996): 'The effect of invivo and invitro non-enzymatic glycosylation and glycoxidation on physicochemical properties of hemoglobin in control and diabetic patients', Int J Biochem Cell Biol., 28, pp. 1343 – 1403

[9] BROWNLEE M., CERAMI A., VLASSARA H. (1988): 'Advanced glycosylation end products in tissue and biochemical basis of diabetic complications', N Eng J Med., 318, pp. 1315 - 1321

[10] MAKITA Z., NAKAYAMA H., TENEDA S., KATO M., KURODA Y., AOKI S., MISAWA K., NAKAGAWA S. (1991): 'Radioimmunoassay for the determination of glycated haemoglobin', Diabetologia, 34(1), pp. 40

[11] SETTER S.M., CAMPBELL R.K., CAHOON C.J. (2003): 'Biochemical pathways for microvascular complications of diabetes mellitus', Am Pharmacother., 37 (12), pp. 1858 – 1866

[12] AVENDANO G.F., AGGARVAL R.K., BASHEY R.L., et al. (1999): 'Effect of glucose intolerance on myocardial function and collagen linked glycation', Diabetes., 48, pp. 1443 -1447

[13] BIERHAUS A., HOFFMANN M.A., ZEIGLER R., NAWROTH., P.P. (1998): 'AGEs and their interaction with AGE-receptors in vascular disease and diabetes mellitus', Cardiovasc Res., 37, pp. 586 - 600

[14] THE EXPERT COMMITTEE. (1999) : 'Report of the Expert Committee on the diagnosis and classification of diabetes mellitus', Diabetes Care., 22, pp. S 5 – S 19

[15] AMERICAN DIABETES ASSOCIATION. (2003): 'Implication of the diabetes control and complication trial. Diabetes Care', 26, pp. S 25 – S 27

[16] EXPERT PANEL ON DETECTION, EVALUATION AND TREATMENT OF HIGH BLOOD CHOLESTEROL IN ADULTS. (2001): 'Executive summary of the third report of the NCEP (Adults Treatment Panel III)', JAMA, 285, pp. 2508 - 2509

[17] FRIEDEWALD W.T., LEVY R.I., FREDRICKSON D.S. (1972): 'Estimation of the concentration of low -density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge', Clin Chem., 18(6), pp. 499 – 502

[18] BIJUKUMAR G., VIGNESHWARAN N., KARMAKAR N., ANAND S., MISRA A. (2003): 'Evaluation of autofluorescent property of Hemoglobin-Advanced Glycation end product as a long term glycemic index of diabetes', Diabetes., 52, pp. 1041 - 1046

[19] STAMOS T.D, AND ROSENSON R.S. (1999): 'Low high-density lipoprotein levels are associated with an elevated blood viscosity', Atherosclerosis, 146, pp. 161 – 165

[20] MAKITA Z., VLASSARA H., RAYFIELD K et al. (1992): 'Hemoglobin-Age: A circulating Marker of Advanced Glycosylation', Science, 258, pp. 651 - 653.

[21] WOLFFENBUTTEL B.H, GIORDANO D., FOUNDS H.W., BUCALA R. (1996): 'Long term assessment of glucose control by hemoglobin- AGE measurement', Lancet, 347, pp. 513 – 515

[22] ROSENSON, R.S., LOWE G.D. (1998): 'Effects of lipids and lipoproteins on thrombosis and rheology', Atherosclerosis, 140, pp. 271 – 280

[23] MELLINGHOFF AC., REINGER A.J., WUERTH J.P., FOUNDS H.W., LANDGRAF R., HEPP K.D. (1997): 'Formation of plasma advanced glycosylation end products (AGEs) has no influence on plasma viscosity', Diabet Med., 14, pp. 832 - 836.

[24] BUHLER I., WALTER R., REINHART W..H. (2001): 'Influence of D- and L- glucose on erythrocytes and blood viscosity', Eur J Clin Invest., 31, pp. 79 – 85

[25] GIUGLIANO D., MARFELLA R. , AND VERRAZO G. et al. (1995): 'Abnormal rheologic effects of glyceryl trinitrate in patients with non-insulin dependent diabetes mellitus and reversal by anti-oxidants', Ann Intern Med., 123 (5) , pp. 338 - 343

[26] MILLER J.A., GRAVALLESE E., BUNN H.F.(1980): 'Nonenzymatic glycosylation of erythrocyte membrane proteins: Relevance to diabetes', J Clin Invest., 65(4), pp. 896-901

[27] KUWAJIMA S. (1993) : 'Immunochemical determination of advanced glycation end products in erythrocyte peripheral-membrane proteins from diabetic patients', Hokkaido Igaku Zasshi., 68 (5), pp. 695 - 704 (English abstract)

[28] HENRY R.R. (2001): 'Preventing cardiovascular complications of type 2 diabetes : 'Focus on lipid management', Clin Diab., 11, pp. 182-189.

[29] BUSTOS P., RADOJKOVIC C., ULLOA N. et al. (2005): 'Lipoprotein composition in children and adolescents with Type 1 diabetes mellitus', J Pediatr Endocrinol Metab.,18 (3 ), pp. 257 -264

[30] CHEN Q.K., CHEN H.Y., WANG L.Y., ZENG Z.Y., ZHOU X.D. (2004): 'Associated risk factors of fatty liver in the patients with type 2 diabetes mellitus', Zhongua Gam Zang


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Bing Za Zhi., 12, pp. 414 -417 (English abstract)

[31] ROSENSON R.S., SHOTT S., LU L., TANGNEY C.C. (2001): 'Hypertriglyceridemia and other factors associated with plasma viscosity', Amer. J Med., 110, pp. 488 - 492

[32] YOKOSE T., AKAYAMA M., MAEDA T., IKEMOTO S., ISOGAI Y. (1985) : 'Hemorheological study in diabetic microangiopathy', Clin Hemor Microcirc., 3, pp. 696 – 700

[33] MISRA A., VIKRAM N.K. (2004) : 'Insulin resistance syndrome (metabolic syndrome) in Asian Indians: Evidence and implications (review)', Nutrition., 20(5), pp. 482 - 491


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