Research Article

ASSOCIATION OF ISCHEMIA MODIFIED ALBUMIN WITH GLYCAEMIC STATUS IN TYPE 2 DIABETES MELLITUS

PriyaPatil[1]., Ashalatha V Rao[2] and Sukanya Shetty[3]

[1,2,3]Biochemistry, KS Hegde Medical Academy, Mangalore. Karnataka

ABSTRACT

Ischemic modified albumin (IMA) has been shown to be a rapidly rising and sensitive biochemical marker especially for the diagnosis of myocardial ischemia. Data exists showing that IMA is also elevated in patients with diabetes mellitus and many other diseases. In view of the possible association between ischemic modified albumin and type 2 diabetes, the present study was taken up to assess the correlation between IMA and glycaemic status in type 2 diabetes mellitus. A total of 90 subjects(45 diabetes subjects and 45 age and sex matched controls) were included. FPG, 2hr-PG, HbA1c were estimated on autoanalyzer and serum IMA was estimated manually. Results show that high serum IMA and HbA1c were observed in diabetic subjects than in controls. The difference was statistically significant. A significant positive correlation between HbA1c and IMA was present. No correlation was present between duration of diabetes with IMA and HbA1c.

INTRODUCTION

Diabetes mellitus (DM) has become a global epidemic. According to the Global prevalence of Diabetes in 2000, there were 171 million adults with diabetes mellitus in the world. It is expected that this number will increase to 366 million by 2030 (*Wild Sarah, 2000*). In India the prevalence of DM is 31.7 million followed by China and USA.

The development of diabetes complications such as cardiovascular diseases and nephropathy is responsible for a significant proportion of the increased death rates in patients with diabetes (*Diabetes care 2009*).

In 2000 the highest number of people with diabetes mellitus were in India (31.7 million) followed by China (20.8 million) and United States (30.3 million) and it was predicted that by 2030, diabetics may increase up to 79.4 million of individuals in India.

***'Criteria for diagnosing diabetes according to American Diabetic Association (ADA) 2015*[18]**

- **Fasting plasma glucose (FPG) ≥ 126mg/dL (7.0 mmol/L)**

Where fasting is defined as no intake of any calorie for ≥ 8 hours.

- 2-hr Plasma Glucose ≥ 200mg/dL (11.1 mmol/L)
- During Oral Glucose Tolerance Test (OGTT) - where a glucose load is 75g.
- HbA1C ≥6.5% (48mmol/mol)
- Random Plasma Glucose ≥ 200mg/dL (11.1mmol/L) in individuals with symptoms of hyperglycaemia or hyperglycaemic crisis'.

Glycated haemoglobin are formed by the nonenzymatic condensation of glucose or other reducing sugars with α and β chain of HbA. The process of glycation of haemoglobin occurs throughout 120 days life span of RBC. Hence, HbA1c level reflects average concentration of blood glucose over previous 3 months and is usually used for monitoring effectiveness of diabetes treatment. Clinically diabetes is defined as high plasma glucose and not by protein glycation, so estimation of HbA1c is not appropriate.

Ischemia-modified albumin (IMA) is a sensitive but nonspecific biomarker for ischemia and oxidative stress as oxidative free radicals can be formed in every kind of ischemia.

Mechanism of IMA generation

Binding of metal ions: The human serum albumin (HSA) N terminal has a binding site for transition metal ions such as cobalt, copper and nickel (*Sadler PJ, 1994*).

Corresponding author: ***PriyaPatil***
Biochemistry, KS Hegde Medical Academy, Mangalore. Karnataka

Many divalent metals bind to HSA in circulation but in Amount far less than that necessary to impact albumin directly. The HSA's N-terminal end is susceptible for biochemical degradation and also less stable compared to the albumin of other species.
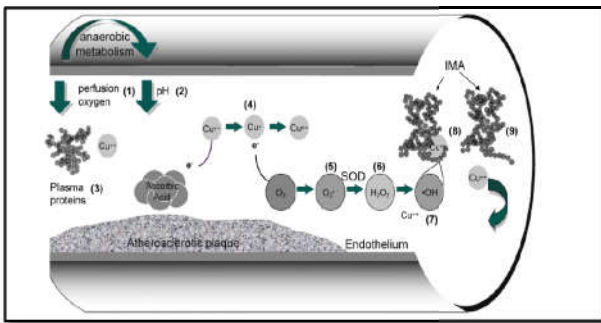

Figure 2 Postulated mechanism of IMA generation

[Ref:Marx, G. and Chevion, M.: Site-specific modification of albumin by free radicals. Reaction with copper (II) and ascorbate. Biochem. J., 236(2): 397–400 (1986)].

The postulate states that the localised ischemia causes acidosis resulting in release of Cu^{+2}ions from the weak binding sites on proteins in circulation. The free Cu^{+2}ions are scavenged by albumin which are tightly bound to N terminus. Free Cu^{+2}can also get reduced to Cu^{+}in presence of reducing agents like ascorbic acid. Cu+2 ions then reacts with O_2 forming Cu^{+2} and producing superoxide free radicals. Superoxide free radicals are converted to H_2O_2 which is degraded by catalase to form OH^- free radicals.OH^- free radicals further damage this copper bound albumin and remove the first three N terminal amino acids and the Cu^{+2} ions are released. The process is repeated and chain continues.

This mechanism is attractive theoretically. However, when sequencing of albumin was done in patients with high IMA levels, expected degradation or truncation of N terminal was not observed.

Binding of fatty acids

Three binding sites for cobalt are identified in human serum albumin, of which two show strong avidity and the binding is not at the N-terminus. Fatty acids can bind the albumin at one of these cobalt binding sites thereby preventing the binding of cobalt.

The estimation of serum IMA was earlier done to study its association with myocardial ischemia. Later, it was investigated in DM as well.

Agnieszka *et al* (2008) were the first to report higher levels of IMA in diabetes patients. Later, Michelle *et al* (2010) observed higher levels of IMA as well as high sensitive C-reactive protein (hs-CRP) in type 2diabetes.Hyperglycaemia and inflammation result in higher IMA levels reducing the capacity of albumin to bind cobalt. In another study, it was found that the baseline IMA levels were significantly higher and positively associated with HbA1c and homocysteine levels in type 2 diabetic patients with peripheral arterial diseases.

Shao-gang *et al* (2012) showed that ischemia modified albumin was significantly associated with diabetic ketosis and was more sensitive than C-reactive protein in reflecting diabetic ketosis.

Dahiya *et al* (2010) observed increased levels of IMA and nitric oxide in diabetic nephropathy with increased degree of proteinuria.

Kurban *et al* (2011) reported a decrease in IMA levels on exercise in type 2 DM. This suggests that regular exercise is beneficial in prevention of oxidative stress in type 2 DM patients.

The aim is to study the association between ischemia modified albumin and glycaemic status in type 2 diabetes mellitus.

MATERIALS AND METHODS

This is a case control study done in the department of biochemistry at K S Hegde Medical Academy, mangalore for a period of 2years.After obtaining approval from the Institutional Ethical Committee, a total of 90 subjects were selected from those attending the out-patient department (OPD) of Medicine at Justice K S Hegde Charitable Hospital, Mangalore.45 of the selected subjects were clinically diagnosed with type 2 DM as per the ADA criteria and remaining 45 were non diabetic at the time of study and considered as controls. Written informed consent was taken from all the subjects included in the study. All the subjects included in the study were aged >40years.Subjects of both genders diagnosed with Type 2 DM were considered. Diagnosis was based as per the recommendations of ADA Criteria 2015.

Subjects with h/o ischemic events or type 2 diabetes with h/o ischemic events. Subjects with liver and kidney dysfunction, infection, corticosteroid therapy, pregnancy. Individuals not willing to participate in the study were excluded from the study.

The general biodata was collected from the study subjects and also anthropometric measurements were taken and BMI calculated.

The biochemical parameters estimated are Fasting plasma Glucose and 2hr- plasma Glucose by Hexokinase method.HbA1c was estimated by Turbidimetric inhibition immunoassay method. TheFPG, 2hr-PG, and HbA1c estimations were performed using autoanalyzer Roche COBAS C311.The Ischemia modified albumin (IMA) was estimated by Albumin cobalt binding test (ACB) *(Bar-Or et al, 2000)*

Statistical analysis

The collected data was analysed by statistical package for social science (SPSS) version 20.Independent Sample t-test was used for studying the significance between the groups.For correlation studies, Karl Pearson Correlation was used.p-Value<0.05 was considered statistically significant.

RESULTS

A total of 90 subjects selected for the study were those attending Justice K S Hegde Charitable Hospital, Mangalore. They were divided into two groups, control group (45) and diabetic group (45). Among the 45 diabetic subjects, 25 (55.4%) were males and 20 (44.6%) females. Among control subjects 23 (50.1%) were males and 22(49.9%) females**.** Among the diabetic subjects, the mean age of male subjects was 56.36± 10.31 years and that of female subjects, 58.5 ± 12.41 years.In control subjects, the mean ages were 56.78 ±

11.09 and 55.73 ± 13.43 years in male and female subjects respectively. The male diabetic subjects had a mean duration of 11.6 ± 7 years and female diabetics that of 12 ± 8.02 years.

Table 1 General characteristics of the study subjects

Parameters	Controls (Mean ± SD)		Cases (Mean ± SD)	
Gender	Male (23)	Female(22)	Male (25)	Female (20)
Age (years)	56.78 ± 11.09	55.73 ±13.43	56.36 ±10.31	58.5 ±12.41
BMI (kg/m^2)	21.07 ± 2.07	24 ± 4.96	24.37± 4.9	25.60 ± 5.29
Duration of DM (years)	-	-	11.6 ± 7	12 ± 8.02

The mean FPG level in the diabetic subjects was 180.7± 57.35 mg/dL and in control subjects, it was 94.53 ± 3.48 mg/dL. The difference between the two groups was statistically significant (p <0.0001), as expected. The overall mean 2hr-PG in the diabetic subjects, 296.60 ± 88.8 mg/dL was expectedly higher than that in controls,166±10.25]]mg/dL (p<0.0001). As expected, the mean HbA1c level in diabetics (8.5 ± 1.9 %) was significantly higher than that (5.2± 0.2%) in the controls (p<0.0001). The mean serum IMA level in the controls was 0.269 ± 0.10 ABSU and that in diabetic subjects was significantly higher i.e. 0.759 ± 0.17 ABSU (p <0.0001) (Table 2). Significantly high serum IMA levels were observed also when the levels in diabetic males and females were compared with the corresponding control groups were considered (0.776± 0.204 ABSU v/s 0.258±0.1 ABSU and 0.737 ± 0.13 ABSU v/s 0.280±0.1 ABSU respectively) (p <0.0001). Among the diabetics group, all subjects had serum IMA values > 0.30 ABSU, 4 subjects between 0.30-0.50 ABSU, 12 subjects between 0.50-0.70 ABSU, 23 subjects 0.70-0.90 ABSU and 6 subjects >0.90 ABSU. Among the control group, 2 subjects had the serum IMA value <0.10 ABSU, 24 subjects 0.10-0.30 ABSU, and 19 subjects 0.30-0.50 ABSU. While the control subjects had the IMA value <0.50 ABSU, most of the diabetic subjects had the IMA value >0.50 ABSU (Figure 1).

Table 2 Biochemical parameters of the study subjects

Parameters	Controls	Diabetic subjects
FPG(mg/dL)	94.53±3.48	180.7±57.35*
2hr- PG(mg/dL)	166±10.25	296.60±88.8*
HbA1C (%)	5.2±0.2	8.5±1.9*
IMA (ABSU)	0.269±0.10	0.756±0.17*

*p value < 0.001
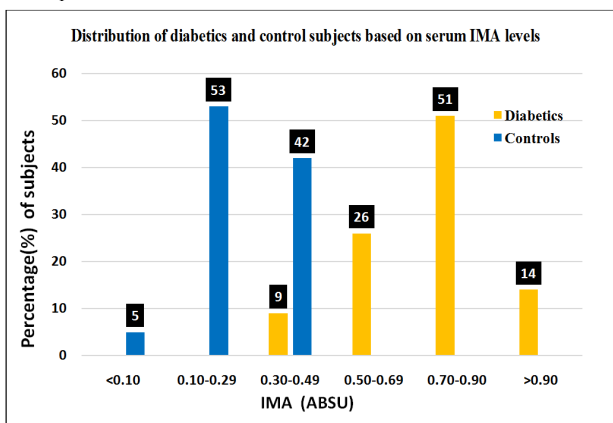
Figure 1 Distribution of diabetics and control subjects based on serum IMA level

Correlation studies

A highly significant correlation between serum IMA and HbA1c was observed with the r value of 0.794 (p< 0.001) (Fig

2)**.** Significant correlations were also observed between IMA and FPG levels (r=0.567) and between IMA and 2hr-PG levels (r value 0.658) (p value <0.001) (Fig 3, 4). No significant correlation was observed between the duration of type 2 DM and either HbA1c or serum IMA (Fig. 5, 6)
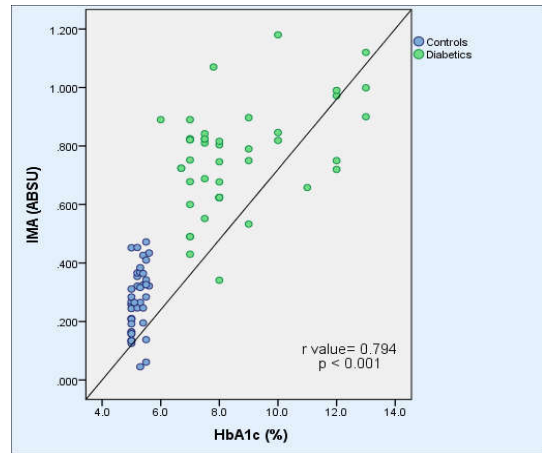
Figure 2 Correlation of IMA and HbA1c in diabetics and control subjects
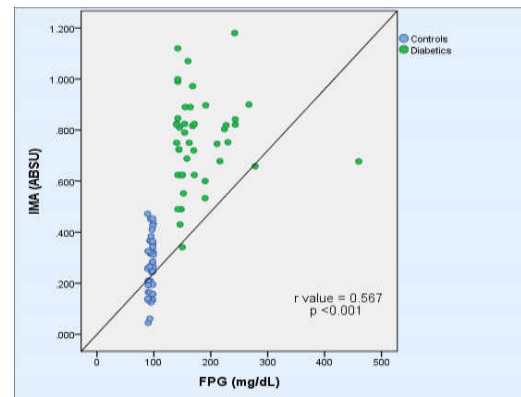
Figure 3 Correlation of serum IMA with Fasting Plasma Glucose levels
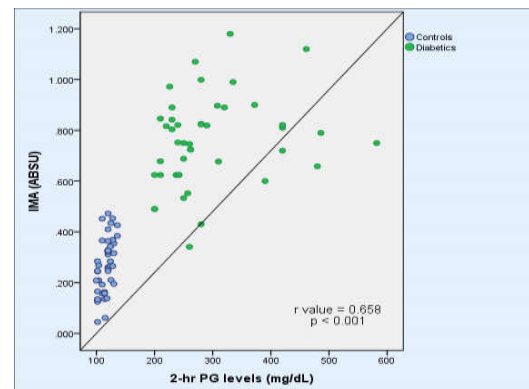
Figure 4 Correlation of serum IMA with 2hr-Plasma Glucose levels

DISCUSSION

It has been observed that occurrence of Type II diabetes mellitus at a much younger age is becoming more common. Mohan *et al* (2007) have found a temporal shift in age at diagnosis of type 2 DM in their Chennai urban rural epidemiology study. 30% of the type 2 DM subjects were in the age group 40-49years and a total of 80% below 60years of age. The DECODA study group have also reported that Indian

subjects have an increased prevalence of impaired glucose regulation in the younger age groups (30-49years). Any such conclusion cannot be arrived at in the present study as only the subjects of age >40years were included. However, when the age at diagnosis of these subjects, based on the duration of diabetes, was considered, it was found that about 70% of subjects were <50 years old. Another observation in the current study was that obesity was present in a higher proportion of diabetic subjects compared to controls.

The fasting and postprandial plasma glucose and HbA1c levels were significantly increased in the diabetic group compared to the control group, as expected.

In patients with DM, the circulating albumin gets continuously exposed to hyperglycaemia and oxidative stress. Hyperglycaemia through various mechanisms like glucose autoxidation, nonenzymaticglycation, polyol pathway, imbalance between the amount of reduced and oxidized coenzyme forms, causes many biochemical sequelae resulting in oxidative stress. This plays a significant role in the destruction of islet cells of pancreas in type 2 DM and development of many late complications.This also causes the oxidative protein damage, formation of advanced glycation end products (AGE's) and probably IMA.

Figure7 Generation of oxidative stress and AGE's due to hyperglycaemia.

[Ref:Michael Brownlee Diabetes 2005; 54:1615 -1625©2005 by American Diabetes Association]

IMA, formed due to structural modification of the circulating albumin, may be formed in diabetes because of the sequelae of oxidative stress and insulin resistance. In the present study, serum IMA was found to be significantly increased in diabetic subjects, both in males and females, compared to control group. This finding is in parallel with those of Piwowar *et al*, Shaogang *et al*, Michelle *et al*, Muhtaroglu *et al*, Wafaa *et al* who reported higher levels of serum IMA in diabetic subjects when compared to controls. Jagiello *et al* observed the increased levels of serum IMA and oxidative stress markers (AOPPs and CML-AGEs) in patients with chronic hepatic C disease with diabetes.

Sowjanya *et al*. observed higher values of IMA (98.94 U/mL) in diabetic patients with complications when compared to those without complication (75.50 U/mL) and control group (51.53 /mL). In the present study, a small number of diabetic subjects (8) had complications other than hypertension. There was not much difference in serum IMA levels between subjects with only diabetes and those with diabetes and hypertension. Although serum IMA levels were higher in diabetic subjects

with other complications when compared to those without complications, no definite conclusions can be drawn as the number of subjects with complications was small.

Significant correlation of IMA with HbA1c, fasting and postprandial plasma glucose was noticed. Similar findings were noted by Chawla *et al*. They also found that the correlation of IMA was better with poor glycaemic control than with good glycaemic control.

CONCLUSION

The mean serum IMA levels were higher in diabetic subjects than in control subjects. A significant positive correlation was observed between IMA and HbA1c, fasting and postprandial plasma glucose. There was no correlation noted between the IMA and duration of diabetes.

At present, estimation of plasma glucose and HbA1c are the investigations of choice for diabetes mellitus. So, as a strong positive correlation between serum IMA levels and HbA1c levels has been observed, it has a potential to be used as an alternative for HbA1C estimation. However, IMA is increased in a number of diseases. Because of this reason its application in diagnosis of any particular disease is hindered. It can only act as an ancillary investigation. Half-life of IMA is same as that of plasma albumin, approximately 21 days. Hence, an advantage of this estimation would be that it reflects average blood glucose level in the previous 20 days unlike that in the previous 3 months as in case of HbA1c.

Limitations of the Study

- Sample size was relatively small
- IMA alone was calculated.IMA to serum Albumin ratio would have been better as it would negate the effect of any alterations in albumin levels

References

Wild Sarah, RoglicGojka, Green Anders *et al*. Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004; 27:1047–1053.

American diabetes association. Standards of medical care in diabetes-2009. Diabetes care 2009; 32:513-61.

Ceriello A. Acute hyperglycaemia: a new risk factor during myocardial infarction. *Eur Heart J*. 2005; 26:328-331.

Kaefer M, Silvia J *et al*. Association between ischemia modified albumin, inflammation and hyperglycaemia in type 2 diabetes mellitus. *Clinical Biochemistry*. 2010 March; 43 (40): 450-454, doi: 10. 1016.

Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial infarction- a prelimary report. *J Emerg Med*. 2000; 19: 311-315.

Piwowar A, Knapik M and Wawas M. Ischemia modified albumin level in type 2 diabetes mellitus-a preliminary report. *Disease Markers*. 2008; 24: 311–317.

Chen CY, Tsai WL, Lin PJ, Shiesh SC. The value of serum ischemia – modified albumin for assessing liver function in patients with chronic liver disease. *ClinChem Lab Med*.2011; 49:1817-21.

Turedi S, Patan T, Gunduz A, Mentese A, Tekinbas C, *et al*. Ischemia-modified albumin in the diagnosis of

pulmonary embolism: an experimental study. *Am J Emerg Med*. 2009; 27: 635–40doi:10.1016/j.ajem. 2008.05.002

Turedi S, Cinar O, Yavuz I, Mentese A, Gunduz A, Karahan SC, *et al*. Differences in ischemia-modified albumin levels between end stage renal disease patients and the normal population. *J Nephrol*.2010; 23:335–40. [Pubmed: 20349416].

Gunduz A, Turedi S, Mentese A, Altunayoglu V, Turan I, Karahan SC, *et al*. Ischemia-modified albumin levels in cerebrovascular accidents. *Am J Emerg Med*. 2008; 26: 874–80.

Seema A K, Jon Cornwakk. The current state of diabetes mellitus in India. *Australian Medical Journal* [AM] 2014, 7(1):45- 48.

Centers for disease control and prevention. National diabetic statistics report: estimates of diabetes and its burden in the United States, 2014. Atlanta, GA: US Department of Health and Human Services.2014 Jul; 2014.

Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes research and clinical practice. 2010 Jan 31; 87(1): 4-14.

American Diabetes Association. Standards of medical care in diabetes- 2015: summary of revisions. *Diabetes Care*. 2015 Jan 1; 38(supplement 1): S4

Brownlee M. The Pathobiology of Diabetic Complications a unifying mechanism. Diabetes [internet]. 2005 Jan 1; 54(6): 1615- 1625. Figure 1, General features of hyperglycaemia- induced tissue damage; pg. 1615.

Samuel rahbar. The discovery of glycated haemoglobin: A major event in the study of nonenzymatic chemistry in biological systems. *Ann. N. Y. Acad. Sci*. 2005, 9(19): 1043.

Wiener K. Varley's Practical Clinical Biochemistry. 6[th]ed. New delhi (India): CBS publishers and distributors:2006. Chapter no 16, Tests in disorders of glucose metabolism; p. 340-341.

Bonora E, Tuomilehto J. The pros and cons of diagnosing diabetes With A1C. *Diabetes Care*. 2011 May 1; 34 (Supplement 2):S184-90.

Prashanth AK, Usha A. Clinical significance of Ischemia Modified Albumin in Critically ill Patients with sepsis. *Ind J Clin Biochem* .Apr- June 2015; 30(2): 194-197

Robertson RP, Harmon J, Tran PO, Poitout V. β-Cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes*. 2004 Feb 1; 53 (Suppl 1):S119–S124**.**

Sadler PJ, Tucker A and Viles, JH. Involvement of a lysine residue in the N-terminal Ni2＋ and Cu2＋ binding site of serum albumins. Comparison with Co2＋, Cd2＋ and Al3＋. *Eur. J. Biochem*. 1994; 220(1): 193–200.

Eltaher WS, Mohamed AS, Mohamed A *et al*. Role of Ischemia Modified Albumin in type 2 Diabetes Mellitus, *Med. J. Cario Univ*. 2016 March;84(2): 29-33.

Sinha MK, Roy D, Gaze DC, Collinson PO, Kaski JC. Role of "Ischemia Modified Albumin", a new biochemical marker of myocardial ischaemia, in the early diagnosis of acute coronary syndromes. *Emergency Medicine Journal*. 2004 Jan 1; 21(1): 29-34.

Liyan C Jie Z, XiaozhouH.Prognostic value of combination of heart- Type Fatty acid binding protein and ischemia modified albumin in patients with acute coronary syndrome and normal troponin-T values. *Journal of clinical laboratory analysis*.2009 Jan 1; 23(1): 14-8.

Roy D, Quiles J, Gaze DC, Collinson P, Kaski JC, Baxter GF. Role of reactive oxygen species on the formation of the novel diagnostic marker ischemia modified albumin. *Heart*. 2006 Jan 1; 92(1): 113-114.

Ma SG, Wei CL, Hong B, Yu WN. Ischemia modified albumin in type 2 diabetic patients with and without peripheral arterial disease. *Clinics*. 2011; 66(10): 1677-80.

Kurban S, Mehmetoglu I, Yerlikaya HF, Gonen S, Erdem S. Effect of chronic regular exercise on serum ischemia modified albumin levels and oxidative stress in type 2 diabetes mellitus. *Endocrine Research*. 2011 Jan 1; 36(3): 116-23.

Wolf HU, Lang W, Zander R. Alkaline haematin D-575, a new tool for the determination of haemoglobin as an alternative to the cyanhaemiglobin method. II. Standardization of the method using pure chlorohaemin. *ClinChimActa* 1984; 136:95-104**.**

Mohan V, Sandeep S, Deepa R *et al*. Epidemiology of type 2 diabetes: Indian scenario. *Indian J Med Res*. 2007 March; 125: p. 217-230

DECODA Study Group. Age and sex-specific prevalence of diabetes and impaired glucose regulation in 11 Asian Cohorts. *Diabetes Care* 2003 Jun 1; 26(6)

****

How to cite this article:

PriyaPatil *et al*.2017, Association of Ischemia Modified Albumin With Glycaemic Status in Type 2 Diabetes Mellitus. *Int J Recent Sci Res*. 8(1), pp. 15374-15378.