Original Article

ID: JCDR/2012/3926:2030

Internal Medicine Section

Serum Adenosine Deaminase Activity and Its Correlation with Glycated Haemoglobin Levels in Patients of Type 2 Diabetes Mellitus

AMANDEEP KAUR, SAHIBA KUKREJA, NARESH MALHOTRA, NEHA

ABSTRACT

Background: Adenosine deaminase (ADA) is suggested to be an important enzyme for modulating the bioactivity of insulin, but its clinical significance in Type 2 diabetes mellitus (Type 2 DM) is not yet characterized. The present study was undertaken to evaluate serum ADA activity and serum uric acid levels in patients of Type 2 DM.

Material and Method: It is a of case control study. The subjects included in this study were divided into 3 groups. Group A consisted of 60 normal healthy individuals who served as controls with no history of DM. Group B consisted of 60 patients of Type 2 Diabetes Mellitus both males & females in the age group of 40-65 years on oral hypoglycaemic drugs with $HbA1_c$ <7%. Group C consisted of 60 patients of Type 2 Diabetes Mellitus both males & females in the age group of 40-65 years on oral hypoglycaemic drugs with $HbA1_c$ >7 %. Serum levels of fasting blood sugar, $HbA1_c$, ADA and uric acid were estimated in all the subjects under study.

Results: All the three parameters, FBS, $HbA1_c$ and ADA levels were found to be increased in the patients of Type 2 DM as compared to controls. The mean serum uric acid levels showed a bell shaped relation with glycaemic control.

Conclusion: From the present study, it is concluded that there is an increase in serum ADA levels with increase in $HbA1_c$ levels. It was found that the serum uric acid levels increased with moderately increasing levels of $HbA1_c$ <7% and then decreased with further increasing levels of $HbA1_c$ >7% (a bell-shaped relation).

Key Words: Diabetes Mellitus type 2, Adenosine deaminase (ADA), Glycated haemoglobin (HbA1c), Fasting blood Sugar (FBS)

INTRODUCTION

Diabetes Mellitus is the most common endocrinological disorder characterized by metabolic abnormalities and long term complications. The incidence and the prevalence of Type 2 DM is globally increasing and becoming a major public health problem for health care providers [1]. Diabetes is projected to increase significantly in the coming period and it is estimated that 80 million people in India would be having diabetes by the year 2030 [2].

Diabetes is a state characterized by chronic hyperglycaemia resulting from diverse aetiologies, environmental and genetic factors acting together. The long term control of diabetes mellitus is judged by glycosylated haemoglobin which was first isolated by Allen et al [3]. It is formed non-enzymatically by a two step reaction. The levels of $HbA1_c$ have increased in diabetic patients and reflected their metabolic control over the past 8-10 weeks [4].

Adenosine deaminase, an enzyme, which is present in red cells and the vessel wall catalyses the irreversible hydrolytic deamination of adenosine to inosine and 2'-deoxyadenosine to 2'-deoxyinosine. Inosine and 2'-deoxyinosine are converted to hypoxanthine, xanthine and finally to uric acid [5]. ADA is considered as a good marker of cell mediated immunity [6]. High lymphocyte ADA activities were found to be elevated in diseases in which there is cell mediated immune response [7].

In a study, Hoshino T et al [5] reported elevated ADA activity in the serum of Type 2 DM patients whereas Angielski S et al [8] demonstrated that 5'-nucleotidase and ADA activities were not changed in isolated glomeruli of streptozocin diabetic rats.

A significant co-relation between the ADA levels and uric acid levels in diabetes was analysed by Kurtul N et al. [9]. They concluded that high uric acid levels in DM patients were due to the increased ADA activity.

Kramer CK et al [10] also reported the association of high uric acid levels with Type 2 DM whereas in a study, Tuomilehto J et al [11] demonstrated low uric acid levels in diabetic patients.

Even though there are reports available on serum adenosine deaminase levels and serum uric acid levels in patients of Type 2 diabetes mellitus but these are still not very clear and conclusive. Moreover, study showing co-relation of serum ADA activity and serum uric acid levels with the glycaemic control in Type 2 DM patients have not been conducted in this part of the country yet.

Hence, in the light of the above mentioned facts, the present study was designed to evaluate the serum ADA activity and serum uric acid levels in patients of Type 2 diabetes mellitus and its comparison with the controls and further to find any correlation of serum ADA activity and serum uric acid levels with the glycaemic control in patients of Type 2 diabetes mellitus.

MATERIALS AND METHODS

The subjects included in the present study were 120 patients of Type 2 diabetes mellitus in age group of 40-65 years of either sex, on oral hypoglycaemic drugs, attending the OPD of Department of Medicine of the institute. A group of 60 normal healthy individuals, age and sex matched from the same population served as controls.

These 180 subjects were divided into 3 groups:

- GROUP A comprised of 60 normal healthy individuals both males and females in the age group of 40-65 years from the general population who volunteered for getting included in the present study.
- GROUP B comprised of 60 patients of Type 2 Diabetes Mellitus both males & females in the age group of 40-65 years on oral hypoglycaemic drugs with HbA1c<7 %.
- GROUP C comprised of 60 patients of Type 2 Diabetes Mellitus age and sex matched on oral hypoglycaemic drugs with HbA1c>7 %.

All the patients and controls were from the same population in the age group of 40–65 years of either sex. Informed consent was taken from all the subjects included in the study. Patients with type 1 diabetes mellitus, acute complications of diabetes mellitus and history of acute infection or other ailments like gross congestive heart failure, tuberculosis, gout, rheumatoid arthritis, skeletal muscle injury and renal failure were not included in this study.

It was a case control prospective study. A complete clinical examination of subjects was done. The subjects were screened for serum blood sugar, serum adenosine deaminase, serum uric acid and glycated haemoglobin. Fasting blood sugar estimation by GOD-POD Method [12]. Glycosylated hemoglobin ($HbA1_c$) estimation by Nycocard Reader [13]. Serum ADA levels estimation by Giusti and Galanti [14]. Serum uric acid estimation by Trivedi R.C. et al. [15].

STATISTICS

Results were analyzed by Oneway ANOVA and Post Hoc Turkey HSD and a probability of less than 5% ($p < 0.05$) was considered to be statistically significant. The study was approved by the ethical committee of the institute.

RESULTS

The statistical analysis showed sex and number distribution in these three groups was comparable [Table/Fig-1]

The mean FBS levels of Group A were 82.00 ± 13.00 mg/dl, Group B were 126.12 ± 22.71 mg/dl and the corresponding values among Group C subjects were 136.97 ± 24.88 mg/dl. In the present study, the mean FBS levels of Group B and Group C were found to be highly significantly higher than Group A ($p < 0.001$). Although the mean FBS levels of Group C were higher than Group B but the difference was statistically not significant ($p=0.115$). It was further observed that the mean $HbA1_c$ levels in Group A were 5.75± 0.46%, in Group B were 6.09± 0.56% and the corresponding values among Group C were 8.72± 1.35%. From this study it was observed that the difference in the levels of $HbA1_c$ was found to be insignificant between Group B and Group A ($p= 0.300$) [Table/Fig-2].

	Group A	Group B	Group C
Number	60	60	60
Male / Female (% age)	53.33/46.66	70/30	43.33/56.66

[Table/Fig-1]: Showing sex and number distribution in three group

In the present study the mean serum ADA levels in Group A were 17.30± 7.28 U/L, in Group B were 30.04± 10.41 U/L whereas in Group C were 44.23± 16.14 U/L. Statistical analysis showed that the mean serum ADA levels of Group C were significantly higher than Group B ($p<0.001$) and the levels of ADA were significantly higher in both Group B and Group C as compared to Group A ($p<0.001$) [Table/Fig-3].

The mean serum uric acid levels in Group A were 6.34 ± 1.62 mg/dl, in Group B were 6.90 ± 1.90 mg/dl and in Group C were 5.13 ± 1.32 mg/dl. The mean serum uric acid levels of Group B were significantly higher than Group C ($p<0.001$) whereas levels of mean serum uric acid in Group C were significantly lower than Group A ($p=0.014$) but no significant difference was observed between Group A and Group B ($p=0.381$) [Table/Fig-4].

The Pearson's correlation coefficient for the relationships between serum ADA, Uric acid and $HbA1_c$ levels in Group B showed positive correlation between $HbA1_c$ and ADA ($r=0.002$). Similarly when the comparison was made between serum uric acid levels and $HbA1_c$ there was positive correlation ($r=0.196$) but when the comparison was made between serum ADA and uric acid there was no correlation ($r=0.000$) [Table/Fig-5].

The Pearson's correlation coefficient for the relationships between serum ADA, Uric acid and $HbA1_c$ levels in Group C showed positive correlation between $HbA1_c$ and ADA ($r=0.125$). When the comparison was made between serum uric acid levels and $HbA1_c$ there was negative correlation ($r= -0.015$) [Table/Fig-5].

DISCUSSION

Diabetes Mellitus is a cluster of abnormal metabolic paradigm having a common feature of hyperglycaemia [16]. Being a chronic metabolic disorder, it has assumed a epidemic proportion and its long term complications could have devastating consequences [17].

Group	No.	FBS			HbA1c		
		Mean ± SD	Comparison	P value	Mean ± SD	Comparison	P value
Group A	60	82.00 ± 13.00	Group A vs. B	<0.001***	5.75± 0.46	Group A vs. B	0.300^{NS}
Group B	60	126.12± 22.71	Group A vs. C	<0.001***	6.09± 0.56	Group A vs. C	<0.001***
Group C	60	136.97± 24.88	Group B vs. C	0.115^{NS}	8.72± 1.35	Group B vs. C	<0.001***

[Table/Fig-2]: Showing FBS & HbA1c in control and study groups
No.: Number of cases; SD: Standard Deviation; $p < 0.001$; Highly Significant.

Group	N	Range (U/L)	Mean ± SD (U/L)	95% CI	Comparison	P value
Group A	60	2.5-30.0	17.30± 7.28	14.58-20.02	Group A vs. B	<0.001***
Group B	60	12.7-55.5	30.04± 10.41	26.16-33.93	Group A vs. C	<0.001***
Group C	60	11.6-82.2	44.23± 16.14	38.21-50.26	Group B vs. C	<0.001***

[Table/Fig-3]: Comparison of Serum Adenosine Deaminase (ADA) levels in three groups
N: Number of cases; SD: Standard Deviation; CI: Confidence Interval;***P<0.001; Highly Significant

Group	N	Range (mg/dl)	Mean ± SD (mg/dl)	95% CI	Comparison	P value
Group A	60	3.8-9.3	6.34 ± 1.62	5.74-6.95	Group A vs. B	0.381^{NS}
Group B	60	4.2-12.9	6.90 ± 1.90	6.19-7.61	Group A vs. C	0.014*
Group C	60	3.3-7.5	5.13 ± 1.32	4.64-5.63	Group B vs. C	<0.001***

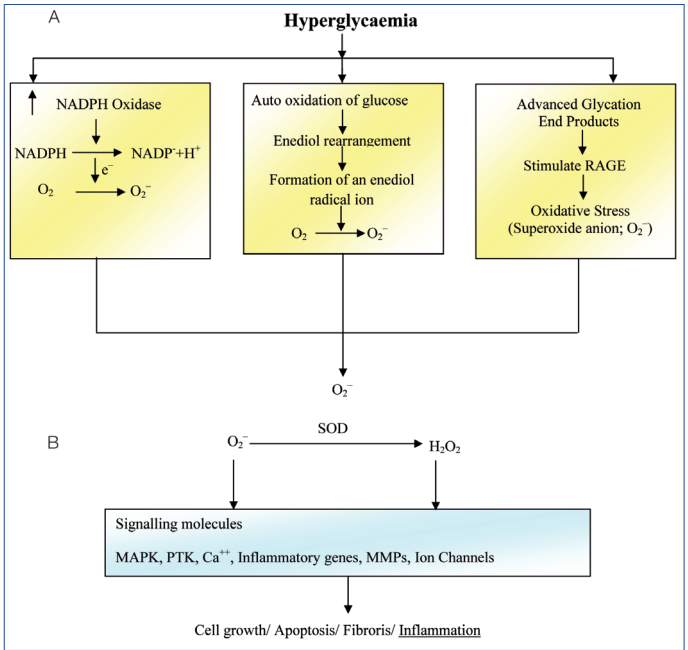
[Table/Fig-4]: Comparison of Serum Uric acid levels in three groups

N: Number of cases; SD: Standard Deviation; CI: Confidence Interval; NS: p > 0.05; Not Significant; *P< 0.05; Significant at 5% significance level; ***P<0.001; Highly Significant.

Parameter		Group B		Group C	
		HbA1c	ADA	HbA1c	ADA
ADA	r value	.002		.125	
	p value	.993		.512	
Uric acid	r value	.196	.000	–0.015	.201
	p value	.300	.998	.939	.287

[Table/Fig-5]: Comparison of Serum ADA, Uric acid and $HbA1_c$ in Group B and Group C.

r value: Pearson correlation coefficient; *Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level.



[Table/Fig-6(A &B)]: Yellow boxes indicate generation of free radicals in diabetic patients. Blue box indicate signalling molecules.

It is characterized by an absolute or relative deficiency of insulin and insulin resistance.

In the present study, we observed that the mean serum ADA levels of Group C were significantly higher than Group B (p< 0.001). Also the levels of ADA were significantly higher in both Group B and Group C than Group A (p < 0.001). Similar results were reported by Hoshino T et al [5] and Kurtal N et al. [9].

Type-2 diabetes mellitus has been shown to be a state of increased free radical activity [18]. Chronic hyperglycaemic status favours auto-oxidation and the formation of advanced glycation end products [19]. The generation of free radicals in the diabetic patients can be due to the following mechanisms.

Hyperglycaemia leads to activation of NADPH oxidase, which is a multi-subunit enzyme, that catalyses $O_2^{-\cdot}$ formation by one electron reduction of O_2 using NADPH or NADH as electron donor [20] [Table/Fig-6(A)].

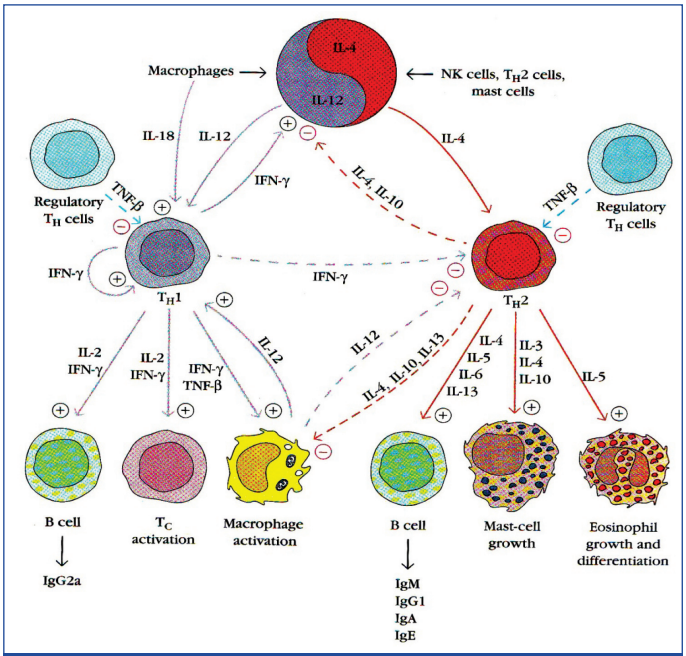
$$2O_2 + NADPH\ (or\ NADH) \rightarrow 2O_2^{-} + NADP\ (or\ NAD) + H^+$$

Another source of superoxide anion formation could be auto-oxidation of glucose which is subjected to enediol rearrangements that result in the formation of an enediol radical ion. This species is capable of reducing molecular oxygen to form superoxide anion[19] [Table/Fig-6(A)].

Hyperglycaemia causes formation of Advanced glycation End Products (AGEs) as result of non-enzymatic reactions between intra-cellular glucose-derived dicarbonyl precursors with the amino group of both intracellular and extracellular proteins [21]. The AGEs stimulate receptors for advanced glycation end products (RAGE). Their interaction is believed to initiate and aggravate the diabetic complications. In addition they increase the generation of reactive oxygen species in macrophages thereby causing heightened oxidative stress [22]. [Table/Fig-6(A)] AGEs bind to AGE receptors on several cell types (endothelial cells, mesangial cells and macrophages) lead to release of cytokines; TNF-α, IL-1, IL-6 and growth factor from macrophages and mesangial cells [23] resulting in activation of T lymphocytes [24] [Table/Fig-7].

Furthermore, in the presence of superoxide dismutase, superoxide anion leads to formation of H_2O_2 which is responsible for activating the signalling molecules leading to inflammation, cell growth, apoptosis and fibrosis [20] [Table/Fig-6(B)].

Activated lymphocytes and macrophages influence each other and also release inflammatory mediators that affect other cells [23]. A close correlation has been found between the severity of inflammation and a local increase in both expression and activity of ADA [25]. ADA plays a crucial role in lymphocyte proliferation and



[Table/Fig-7]: The regulation of T_H subsets by cytokines. Solid arrows indicate stimulatory effects (+); dashed arrows indicate inhibitory effects (–). Purple arrows indicate the cytokines of the T_H1 pathway; orange, the T_H2 pathway.

differentiation [26] and shows its highest activity in T- lymphocytes [6]. The high plasma ADA activity might be due to abnormal T-lymphocyte responses or proliferation [26].

The Pearson's correlation coefficient for the relationships between serum ADA, Uric acid and $HbA1_c$ levels in Group B showed positive correlation between $HbA1_c$ and ADA (r=0.002) but the difference was not statistically significant (p=0.993) which means that with the increase in $HbA1_c$, levels of serum ADA were also increased. This finding was in accordance with the study of Kurtul N et al. [9]. Similarly when the comparison was made between serum uric acid levels and $HbA1_c$ there was positive correlation (r=0.196) which was statistically not significant (p=0.300). The reason for increased uric acid levels in this group could be due to increased activity of ADA, an enzyme responsible for converting adenosine to uric acid. Another reason behind the increase in serum uric acid levels could be due to increased flux of glucose-6-phosphate through the hexose monophosphate shunt due to impairment of the glycolytic pathway. This finding was in accordance with study by Dehghan A et al [27] and Modan M et al. [28].

The Pearson's correlation coefficient for the relationships between serum ADA, Uric acid and $HbA1_c$ levels in Group C showed positive correlation between $HbA1_c$ and ADA (r=0.125). Although statistically not significant (p=0.512). Similarly when the comparison was made between serum ADA and uric acid there was positive correlation (r=0.201) but statistically not significant (p=0.287). When the comparison was made between serum uric acid levels and $HbA1_c$ there was negative correlation (r=-0.015) which was statistically not significant (p=0.939) meaning thereby that when the levels of $HbA1_c$ increased more than 7% there is decrease in uric acid levels. The reason for this finding is thought to be due to the uricosuric effect of glycosuria. This finding was in accordance with Choi H.K. et al [29] and Tuomilehto J et al study [11].

In conclusion our study suggests that there is an increase in serum ADA levels with increase in $HbA1_c$ levels. Furthermore, it was found that the serum uric acid levels increased with moderately increasing levels of $HbA1_c$ (<7%) and then decreased with further increasing levels of $HbA1_c$ >7% (a bell-shaped relation). Serum ADA and serum uric acid levels reflect closely related components of the same disease i.e. Type 2 Diabetes Mellitus.

ACKNOWLEDGEMENT

The authors are thankful to the management of the institute for the financial support.

REFERENCES

[1] Sheetz MJ, King GL. Molecular understanding of hyperglycemia's adverse effects for diabetic complications. *JAMA* 2002; 288: 2579-88.
[2] Wild S, Roglic G, Green A, et al. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; 27: 1047-53.
[3] Allen DW, Schroeder WA and Balog. Observation on the chromatographic heterogenicity of normal adult and fetal Hb: a study of the effect of crystallization and chromatography as the heterogenicity and isoleucine content. *Am J Chem Soc* 1958; 80: 1628-34.
[4] Santigo JV, Davis JE and Fisher F. Haemoglobin $A1_c$ levels in diabetic detection. *J Clin Endocrinol* 1978; 47: 578-81.
[5] Hoshino T, Yamada K, Masuoka K, et al. Elevated adenosine deaminase activity in the serum of patients with DM. *Diabetes Res Clin Pract* 1994; 25: 97-102.
[6] Sullivan JL, Oxborne WRA, Wedgewood RJ. Adenosine deaminase activity in lymphocytes. *Br J Haematol* 1977; 37: 157-58.
[7] Prakash MS, Chennaiah S, Murthy YSR, et al. Altered adenosine deaminase activity in Type 2 diabetes mellitus. *JIACM* 2006; 7(2): 114-17.
[8] Angielski s, Jakubowski Z, Pawelczyk T, Piec G, Redlak M. Renal handling and metabolism of adenosine in diabetic rats. *Contrib Nephrol* 1989; 73:52-58.
[9] Kurtul N, Pence S, Akarsu E, et al. Adenosine deaminase activity in the serum of type 2 diabetic patients. *Acta Medica* (Hradec Kralove) 2004; 47 (1): 33-35.
[10] Kramer CK, Muhlen DV, Jassal SK and Connor EB. Serum uric acid levels improve prediction of incident Type 2 Diabetes in individuals with impaired fasting glucose. *Diabetes Care* 2009; 32: 1272-73.
[11] Tuomilehto J, Zimmet P, Wolf E, Richard T, Ram P and King H. Plasma uric acid level and its association with Diabetes Mellitus and some biologic parameters in a biracial population of Fiji. *American Journal of Epidemiology* 1987; 127(2): 321-36.
[12] Trinder P. Blood sugar estimation by GOD-POD method. *Ann. Clin. Biochem*.1969; 6: 24-27.
[13] Jeppson JO. Approved IFCC Reference Method for the measurement of $HbA1_c$ in human blood. *Clin Chem Lab Method* 2002: 40(1): 78-89.
[14] Giusti G. Adenosine deaminase. Methods of enzymatic analysis. In: Bergmeyer HU editor 2nd ed. New York: Academic press inc 1974; 2: 1092-99.
[15] Trivedi RC, Rebar L, Berka E, Strong L. Enzymatic calorimetric method of uric acid determination. *Clin. Chem*. 24, 1978.
[16] Stymvoli M, Goldstecn B, Haeften TW. Type 2 diabetes: Principles of pathogenesis and therapy. *Lancet* 2005; 365: 1333-45.
[17] Bona KS, Belle LP, Sari MH, Thome G, Schetinger MRC, Morsch VM, et al. Syzygium cumini extract decrease Adenosine deaminase, 5' nucleotidase activities and oxidative damage in platelets of diabetic patients. *Cell Physiol Biochem* 2010; 26: 729-38.
[18] Ankush RD, Suryakar AN, Ankush, NR. Hypomagnesaemia in Type-2 Diabetes Mellitus patients: A Study on the status of oxidative and nitrosative stress. *Indian Journal of Clinical Biochemistry* 2009; 24(2): 184-89.
[19] Geetanjali G, Sudeep G, Neerja, Mili G, Deepak A, Priyanka S. The effect of hyperglycaemia on some biochemical parameters in diabetes mellitus. *Journal of Clinical and Diagnostic Research* 2010; 4: 3181-86.
[20] Singh PP, Mahadi F, Roy A and Sharma P. Reactive oxygen species, reactive nitrogen species and antioxidants in etiopathogenesis of diabetes mellitus type -2. *Indian Journal of Clinical Biochemistry* 2009; 24(4): 324-42.
[21] Kumar V, Abbas AK, Fausto N. The endocrine system. *Robbins and Cotran Pathologic basis of disease* 7th ed; 2008. p. 1198.
[22] Ahmed N. Advanced glycation end products role in pathology of diabetic complications. *Diab Res Clin Pract* 2005; 67: 3-21.
[23] Kumar V, Abbas AK, Fausto N. The endocrine system. *Robbins and Cotran Pathologic basis of disease* 7th ed; 2008. p. 82.
[24] Goldsby RA, Kindt TJ, Osborne BA. Cytokines. Kuby immunology. 4th ed. New York: W.H. Freeman and Company; 2000. p. 320.
[25] Desrosiers MD, Cembrola KM, Fakir MJ, Stephens LA, Jama FM, Shameli A, et al. Adenosine deamination sustains dendritic cell activation in inflammation. *The Journal of Immunology* 2007; 179: 1884-92.
[26] Hovi T, Smyth JF, Allison AC, Williams SC. Role of adenosine deaminase in lymphocyte proliferation. *Clin Exp Immunol* 1976; 23: 395-403.
[27] Dehghan A, Van Hoek M, Sijbrands EJG, Hofman A, and Witteman JCM. High serum uric acid as a novel risk factor for type 2 diabetes. *Diabetes Care* 2008; 31: 361–62.
[28] Modan M, Halkin H, Karasik A and Lusky A. Elevated serum uric acid – a facet of hyperinsulinaemia. *Diabetologia* 1987; 30: 713-18.
[29] Choi HK and Ford ES. Haemoglobin $A1_c$, fasting glucose, serum C-peptide and insulin resistance in relation to serum uric acid levels — the Third National Health and Nutrition Examination Survey. *Rheumatology* 2008; 47(5): 713-17.

AUTHOR(S):

1. Dr. Amandeep Kaur
2. Dr. Sahiba Kukreja
3. Dr. Naresh Malhotra
4. Dr. Neha

PARTICULARS OF CONTRIBUTORS:

1. Junior resident, (Corresponding Author)
2. Associate Professor,
3. Head and Professor,
4. Assistant Professor.

NAME OF DEPARTMENT(S)/INSTITUTION(S) TO WHICH THE WORK IS ATTRIBUTED:

Deparment of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Amandeep Kaur
F- 7/65, Kashmir Avenue West, Amritsar, Punjab, India.
Phone: 09915922294
E-mail: amandeep_best@yahoo.com

FINANCIAL OR OTHER COMPETING INTERESTS:

None.

Date of Submission: **Jan 06, 2012**
Date of peer review: **Feb 09, 2012**
Date of acceptance: **Feb 21, 2012**
Date of Publishing: **Apr 15, 2012**