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HYPOMAGNESEMIA A COFACTOR FOR ATHEROGENESIS IN NON INSULIN DEPENDENT DIABETES MELLITUS

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ABSTRACT

To investigate correlation between Hypomagnesemia and biochemical parameters of atherosclerosis in NIDDM 2.To investigate correlation between serum "C"-peptide levels and glycemic control as evaluated by HbA1c.ASRAM college of Medical Sciences & ASRAM Hospital outpatients and inpatients, Eluru,AP .76 patients of Type II Diabetes Mellitus(NIDDM) aged above 40yrs & 30 number of age & sex matched healthy controls.Serum C-peptide was measured by Direct chemiluminiscence. Fasting Blood Glucose, HbA1c, Serum Total Cholesterol, HDL-C,and Serum Magnesium were considered for analysis and measured using standardized kits from 'Coral clinical systems'.The optical density readings taken in a photoelectric colorimeter.Patients with Hypomagnesemia defined as serum Mg++ <1.6 mg/dl were compared with those having normal serum Mg++ levels (1.6-3.0mg/dl).In the present study inverse relation observed between glycemic control and serum Magnesium levels P-<0.0034.The serum C-Peptide levels showed greater variance in hypomagnesemic diabetic patients compared to normomagnesemic diabetic patients (P <0.012).Strong correlation observed between serum C-Peptide levels and glycemic control with reduced C-Peptide levels in diabetic patients with poor glycemic control (>8%). P<0.00001,The presence of associated complications in diabetic patients showed reduced C-peptide levels,(P-value <0.00001).ANOVA between controls and diabetic patients with hypomagnesemia showed significantly elevated TC/HDLC ratio in diabetic patients with hypomagnesemia (P-<0.026).The present study concluded with the principal observation that glycemic control and reduced C-Peptide levels influenced hypomagnesemia.Deleterious effects of dyslipidemia of Diabetes Mellitus per se were exacerbated by hypomagnesemia and reduced C-Peptide levels.

KEY WORDS: NIDDM, Hypomagnesemia, Glycemic control, C-peptide, atherosclerosis

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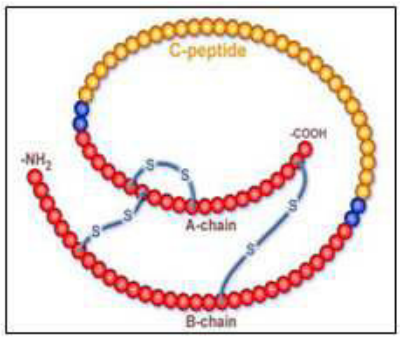
INTRODUCTION

Metabolic disorders of NIDDM include abnormalities of carbohydrates, proteins and lipids. In addition there is hypokalemia, hypocalcemia and hypomagnesemia.Hypomagnesemia has negative impact on glucose homeostasis, insulin sensitivity and complications.[30] To meet the energy requirements lipolysis increases with resultant hyperlipidemia especially of Non-Esterfied Fatty Acids, Triacylglycerol (TAG) and cholesterol. When the pancreas produces Proinsulin, it splits into Insulin and C-peptide. C-peptide binds to receptor at the cell surface, activates G-protein coupled receptor and increases intracellular calcium which activates intracellular signal transduction pathways (PKCα, MAPK, ERK) that result in stimulation of Na^+/ K^+ ATPase and endothelial Nitric Oxide synthase. (eNOS)[29]. C-peptide induces smooth muscle cell proliferation through phorsphorylation of the protein tyrosine kinase src[25]. Hypomagnasemia of Diabetes Mellitus is secondary to osmotic diuresis, metabolic acidosis causing retention of Magnesium in cells, reduced renal reabsorption of Magnesium in the Thick Ascending Limb of Loop of Henle and decreased Gastro- intestinal absorption of Magnesium. Microvascular changes in chronic diabetes with or without hypertension due to Hypomagnesemia with increased Low Density Lipoprotein – Cholesterol are factors for thickening of vascular basement membrane, cellular growth and death and increased vascular permeability[3]. Hypomagnesemia causes vasospasm and increased vasoconstrictor activity, elevation of smooth muscle Calcium concentration, cardiac intracellular Calcium concentration[26], the formation of free radicals, proinflammatory agents and growth factors along with changes in membrane permeability and transport[8]. Persistent hyperglycemia causes Hypomagnesemia. Hypomagnesemia elevates serum Total Cholesterol and Triacylglycerol. Total cholesterol to High Density Lipoprotein-Cholesterol ratio also increases. These changes cause an increase in vasospasm and cardiac contractility. Hypomagnesemia is a contributing factor to the complications associated with dyslipidemia and electrolyte imbalance of Type II Diabetes Mellitus. Insulin resistance in NIDDM is associated with decreased number of insulin receptors. Post receptor signaling by insulin is impaired. Insulin resistance regardless of its mechanism results in the inability of circulating insulin to properly direct the deposition of glucose, a more persistent hyperglycemia and more prolonged stimulation of pancreatic β - cell.

Glycosylated hemoglobin[5]

Measurement of glycated proteins is useful in monitoring long term glucose control in diabetes mellitus. It provides a retrospective index of the integrated Plasma glucose values over an extended period of time and is not subject to the wide fluctuations observed when assaying blood glucose levels. HbA1c is formed by condensation of glucose with the N-terminal Valine of each β-chain of HbA to form an unstable Schiff base – aldimine preA1c, which then undergoes Amadori rearrangement to form a ketamine, HbA1c.

C-PEPTIDE (CONNECTING PEPTIDE)



FUNCTIONS

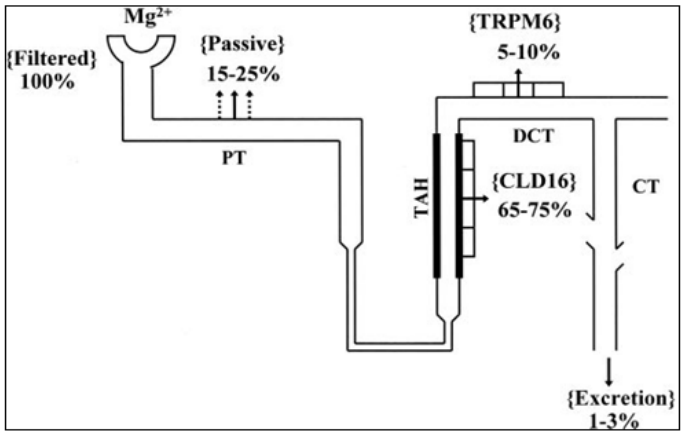
1. Improves glycemic control.[12]
2. Suppresses diabetic induced abnormal glomerular eNOS Expression.[16]
3. Prevents diabetic nephropathy.[19] Reduces microalbuminuria.[12] Reduces diabetes induced glomerular hyperfiltration.[12]
4. Improves coronary vascular flow.
5. Improves endoneural blood flow.[7]
6. In the presence of insulin resistance and hyperinsulinemia, induces smooth muscle cell proliferation.[19]Helps in repair of muscular layer of arteries.[6]

Hypomagnesemia and Diabetes Mellitus

Non-insulin dependent Diabetes Mellitus is one of the most common causes of Magnesium deficiency with an incidence of hypomagnesemia between 25% - 39%. The serum Magnesium concentration is correlated with fasting blood sugar and glycated hemoglobin. Grafton and Baxter[9] suggested Hypomagnesemia leads to reduction of Inositol triphosphote (IP_3) diffusion into cells and subsequent inositol depletion leads to hypocalcemia and enhances the development of diabetic complications like retinopathy and hypertension.

Magnesium and cell physiology

Magnesium is fourth most abundant cation in human body and second most abundant intracellular cation. The adult human body (70kg B.W) contains approximately 21 – 28 gms of Magnesium. Out of the total body magnesium about 60% is in bone, 20% in skeletal muscle, 19% in other cells and about 1% in extra cellular fluid.[19] The major roles for Magnesium in biological systems (1) It can compete with Calcium for binding sites on proteins and membranes. (2) It can form chelates with important anionic ligands notably ATP (3) It serves as a co-factor for all enzymatic reactions that require ATP[20]. (4)It is also an enzyme activator for neuromuscular excitability and cell permeability (5) A regulator of ion channels and mitochondrial function[27] (6) A critical element in cellular proliferation and apoptosis.(7) It is an important factor in both cellular and humoral immune reactions[2]. Mg^{++} is involved in hormone receptor binding and gating of Ca^{+2} channels, transient ion flux regulation of adenylate cyclase, muscle contraction and neuronal activity, control of vascular tone, cardiac excitability and neurotransmitter release[22]. Mg^{++} increases body's ability to utilize Ca^{+2}, Na^{+}, K^{+} and Vitamin C, E and B-Complex.[17] Normal plasma Mg^{++} levels in health are 1.7 to 2.4 mg/dl or 0.7mmol – 1.0 mmol/L. Low Mg^{++} levels may promote endothelial cell dysfunction and thrombogenesis via increased platelet aggregation and vascular calcifications.



Mg++handling at various levels in a Nephron

Hypomagnesemia also induces proinflammatory and profibrinogenic response, reduction of protection enzymes against oxidative stress[23], induction or augmentation of vasoconstriction and hypertension[4] and stimulation of aldosterone[10]. Mg^{++} deficiency interferes with normal cell growth and regulation of apoptosis.

Diabetic Vasculopathy

Diabetic Vasculopathy is caused by enhanced vascular oxidative stress and generation of ROS (Reactive Oxygen Species)[24], formation of advanced glycation end products (AGEs), activation of DAG- PKC, expression and action of vascular mediators and flux through polyol pathway[11.] HbA1c was shown to have affinity for oxygen thereby causing tissue anoxia and microangiopathy. The interaction of AGEs & their receptorshave been implicated as mediators of microvascular permeability, ischemia and angiogenesis.[30] Grafton and Braxtor[9] observed reduction of inositol transport secondary to hypomagnesemia. Subsequent hypoinositol enhances development of diabetic complications.

MATERIALS AND METHODS

From ASRAM Hospital and Medical College 76subjects belonging to Type II Diabetic patients above 40 yrs were selected for this study after obtaining informed written consent. 30 number of healthy controls belonging to the same age and sex selected.

Exclusion criteria

a) Endocrine disorders other than Diabetes Mellitus like cushing's syndrome, Thyroid abnormalities.
b) Gestational Diabetes Mellitus.
c) Renal pathologies other than those secondary to Diabetes Mellitus.
d) Use of diuretics & drugs which cause hypomagnesemia (Ex: Cisplatin)

Procedure for Sample Collection & evaluation

Fasting blood sample was collected under strict aseptic conditions.

EDTA Sample for estimation of Hb A1c. by Ion exchange resin method.

Serum separated for estimation of fasting blood glucose (FBG), cholesterol, HDL – C, Magnesium and C-peptide. All the parameters evaluated by kit method using standardized kits from 'Coral clinical systems'. The optical density readings taken in a photoelectric colorimeter.

Serum Magnesium evaluated by Calmagite method.

Serum Total Cholesterol estimated Cholesterol Oxidase-Peroxidase method.

Serum HDL-C estimated by Cholesterol Oxidase-peroxidase method after precipitating chylomicrons, LDL and VLDL

Serum FBS evaluated by Glucose- oxidase peroxidase method.

Serum C-peptide evaluated by Direct chemiluminiscence.

RESULTS

Statistical analysis carried out using SPSS for windows version-16(2007). Data expressed in Mean ±S.D., one-way ANOVA, Student 't' test & 'chi' square test.

Table I
ESTIMATED MEAN SERUM LEVELS OF FBS, HbA1c, MAGNESIUM, C-PEPTIDE AND CHOLESTEROL/HDLC RATIO IN CONTROLS

	FBS Mg/dl	HbA1c %	Mg++ Mg/dl	TC Mg/dl	HDLC Mg/dl	TC/HDLC	C-Peptide Ng/ml
Normal range	**70-110**	**4%-7%**	**1.6- 3.0**	**150-200**	**35-60**	**<4.5**	**0.9-7.1**
Mean	85.8	4.907	1.82	198.3	45.05	4.47	5.9
S.D.±	7.27	1.023	0.13	34.97	7.47	1.07	0.9

Table II A
THE ESTIMATED MEAN SERUM LEVELS OF FBS, HB A_1C AND MAGNESIUM IN TYPE II DEABETIC PATIENTS

Parameter	FBS mg/dl	Hb A_1C %	Mg^{+2} mg/dl
Normal range	**70-110**	**4% -7%**	**1.6 – 3.0**
Type II DM	135.9 ± 59.94	6.95 ± 2.85	1.25 ±0.49

The estimated mean Hb A_1c percentage in TypeII DM were in the range of 6.95 ±□2.85% indicates a fair glycemic control.
The estimated mean serum Mg^{++} levels in Type II DM were 1.25 ±□0.49 mg/dl, showed definite hypomagnesemia.
Table II A shows that the estimated mean serum FBS levels were in the range of 135.9 ±□59.94 mg/dl.

Table III
ESTIMATED MEAN SERUM MAGNESIUM LEVELS IN CONTROLS AND DIABETES PATIENTS WITH HYPOMAGNESEMIA

	Controls	**Diabetes patients with hypomagnesemia**
Mean (mg/dl)	2.49	1.05
SD	± 0.38	±0.32
SEM	0.08	0.04
P-Value		<0.000001
Significance		Highly significant

Table IV
ANOVA OF HbA1c BETWEEN CONTROLS AND DIABETES PATIENTS WITH HYPOMAGNESEMIA

	Controls	**Diabetes patients with hypomagnesemia**
Mean %	4.93	6.56
SD	±1.10	±3.03
SEM	0.23	0.44
P.Vlaue		< 0.0034

Statistically significant.

Table III shows the estimated mean serum Mg++ levels in controls and in diabetic patients with hypomagnesemia to be in the range of 2.49 ±0.38mg/dl and 1.05 ±□0.32mg/dl respectively. According to the results the levels of serum magnesium were significantly reduced in diabetic patients as compared to healthy subjects (P<0.000001). The study is in accordance with that of C.P.Hans, R. Sialy, D.D. Bansal et al [31].Table IV shows ANOVA of HbA_1c between controls and diabetes patients with hypomagnesemia. The estimated mean HbA_1c in controls and diabetic patients with hypomagnesemia were 4.93 ±1.10 and 6.56 ±3.03 respectively. The P value<0.0034 statistically highly significant, in accordance with that of Linda W.H.,Folsom A.R.et.al.[32]

Table V
ANOVA OF TC/HDL-C BETWEEN CONTROLS AND DIABETES PATIENTS WITH HYPOMAGNESEMIA

	Controls	**Diabetes patients with hypomagnesemia**
Mean	4.20	4.21
SD	±0.32	±0.57
SEM	0.06	0.08
P.Vlaue		<0.026

Statistically significant

Table V shows ANOVA of TC/HDL-C ratio between controls and diabetes patients with hypomagnesemia. The estimated mean TC/HDLC ratio in controls and diabetes patients with hypomagnesemia were 4.2 ±□0.32 and 4.21 ±0.57 respectively. The P.Value <0.026 statistically significant, in accordance with that of Gupta B.K. et.al.[23]

Student 't' test

Correlation of c-peptide and Glycemic control (HbA1c) in DM patients

Table VI

	Good Glycemic Control	Poor Glycemic Control
N	32	44
Mean ng/ml	3.864	2.254
S.D.	1.428	1.469
SE Mean	0.220	0.212
P-value		**< 0.00001**

"Chi" square test to detect variance in C-Peptide levels in normo magnesia and hypomagnesia of Type II diabetes mellitus.

Type II diabetics with Hypomagnesemia (1)	Type II diabetics with Normomagnesemia (2)
N 55	**19**
χ^2 **83.632**	**30.806**

$$\text{Variance ratio (F)} = \frac{\chi.^2(1)}{\chi.^2(2)} = \frac{83.632}{30.806} = 2.714$$

Df = 54 & 18 from the table
For dr of 54, 18 from the table
The value obtained in test 2.714 is marginally higher that calculate value.
P-value is <0.012. Statistically significant.
C-Peptide levels show greater variance in hypomagnesemia to normomagnesemia in Type II Diabetes[13.]

A total number of 106 subjects included in the present study. They were classified into controls numbered 30 and 76 NIDDM patients. Among 76 subjects 41 were males and 35 were females.

SERUM MAGNESIUM

The estimated mean serum Mg^{++} levels were in the range of 2.49±0.38mg/dl, 1.05±0.32mg/dl and 2.01±0.34mg/dl in controls, in diabetic patients with hypomagnesemia and in diabetic patients with normomagnesemia respectively. P-value<0.000001.This study correlated with the study of Puong-Chi T. Pham, Puong – Mai T. Pham Son V Pham Jeffery M Miller[14]. The ANOVA of HbA_1c between controls and diabetics with hypomagnesemia found to be 4.9 ±1.10 and 6.5± 3.03 respectively with P-value < 0.0034 (Table IV) were highly significant. This finding correlated with that of American Diabetic Association guidelines.[1] In 32 diabetic subjects with good glycemic control (HbA1c 4% - 7%) the mean C-Peptide values were in the range of 3.86 ± 1.42.ng/ml. In 44 subjects with poor glycemic control, (HbA1c >8%) C-Peptide levels were in the range of 2.254±1.46ng/ml.P-value <.0.00001. (Table VI) ARIC study demonstrated strong cross-sectional association between serum Magnesium levels and Type II diabetes[32] correlated with the study of Sjoberg S[12] et al. Hypomagnesemia of Diabetes Mellitus causes elevation of total Cholesterol and consequently increases TC/HDLC ratio as observed by Bussiere L, Mazurr,A. Gueux,E and Rayssiguier.[3] This study proved the observation as evidenced by ANOVA between controls 4.2±0.32 and diabetic patients with hypomagnesemia 4.21±0.57 significant P-value <0.026.(Table V) "Chi" square test of C-Peptide levels show greater variance in

hypomagnesemia to normomagnesemia in Type II Diabetes.13 (P-value is < 0.012.)

DISCUSSION

In the present study inverse relation observed between glycemic control and serum Magnesium levels P-<0.0034. This correlated with that of American Diabetic Association guidelines.[1] The serum C-Peptide levels showed greater variance in hypomagnesemic diabetic patients compared to normomagnesemic diabetic patients (P <0.012). Substantiating evidence to this found in International Text Book of Diabetes Mellitus III Ed[11] Strong correlation observed between serum C-Peptide levels and glycemic control with reduced C-Peptide levels in diabetic patients with poor glycemic control (>8%). P<0.00001, correlated with study of Sjoberg et al.[6] ANOVA between controls and diabetic patients with hypomagnesemia showed significantly elevated TC/HDLC ratio in diabetic patients with hypomagnesemia. (P-<0.026) correlated with study of Bussiere L, Mazur A, Gueex E and Rayssiguier[3] observed the same. The presence of associated complications in diabetic patients showed reduced C-peptide levels, P-value <0.00001. C-Peptide levels indicate endogenous insulin secretion and responsible for Magnesium homeostasis. The reduced C-peptide concentrations Type II diabetes exacerbate hypomagnesemia by reducing Na^+/K^+ ATPase activity in PCT and TAL of Henle. Further hypomagnesemia also induces oxidative stress by increasing free radical formation.

CONCLUSION

Persistent hyperglycemia causes Hypomagnesemia. Hypomagnesemia elevates serum Total Cholesterol and Triacylglycerol. Total cholesterol to High Density Lipoprotein-Cholesterol ratio also increases. These changes cause an increase in vasospasm and cardiac contractility[28]. Hypomagnesemia is a contributing factor to the complications associated with dyslipidemia and electrolyte imbalance of Type II Diabetes Mellitus. Microvascular changes in chronic diabetes with or without associated hypertension due to Hypomagnesemia with increased Low Density Lipoprotein – Cholesterol are factors for thickening of vascular basement membrane, cellular
growth which leads to increased vascular permeability[3,] endothelial dysfunction causing atherogenesis.

REFERENCES

1. American Diabetic Association, Diab.Care.1992 15. 1065-1067
2. Althura B.M. Basic Biochemistry and Physiology of Mg. A Brief review. Mf. and trace elements 1991: 10: 167-171.
3. Bussiere, L. Mazur, A. Gueux. E. and Rayssinguier. Y.J. Nutr. Biochem 1994, 5, 585-590.(http://www.sciencedirect.com /science/preview/abstract)
4. Chakraborti S, Chakraborti. T, Mandal M, Mandal A, Das S, Ghosh S, Protective role of Mg: in cardiovascular diseases: A review. Mol Cell Biochem. 238:1315, 1984.
5. Clinical chemistry, Theory analysis, correlation, 4th edition, Lawrence A. Kaplan Amadeo J. Pesce, Steven C Kazmierczak. Page 592-593.
6. C-Peptide Exerts Beneficial effects on Myocardial Blood Flow and Function in Patients With Type 1 Diabetes – Hansen et al. 51 (10): 3077 – Diabetes
7. C-Peptide Reverses Nociceptive Neuropathy in Type 1 Diabetes – Kamiya et al. 55 (12): 3581 – Diabetes
8. Cholst I.N., Steinberg.S.F.,Tropper P.J.,Fox.H.E, Segre.G.V., and Bilezikan.J.P.N.Engl.J.Med., 1984, 310, no. 19,1221-1225.(www.pdiconnect.com)
9. Grafton.G. and Baxter, M.A.J. Diab complications, 1992, 6, 143-149.(www.scribd.com/doc/33757155/Magnesium)
10. Ichihara A, Suzuki H, Saruta T : Effects of Mg. on the rennin-angiotension-Aldosterone system in human subjects J. Lab clin Med. 122 : 432-440, 1993.

11. International text book of Diabetes Mellitus- III edition, Vol.1, pages 4 -7 & pages 165 - 175
12. Johansson B L Kernell A, Sjoberg S, Wahren J Differential effects of proinsulin C-Peptide fragments on Na+ K+ATPase activity of renal tubule segments. Diabetalgia 1998;41: 287-91.
13. Johansson B-L Kernell A, Sjoberg S, Wahren J. Influence of combined C-Peptide and insulin administration on renal function and metabolic control in diabetes type 1. Journal of Clinical Endocrinology & Metabolism 1993; 77(4); 976-981. [pubmed]
14. Puong-Chi T. Pham, Puong-Mai T. Pham son V Pham Jeffery M Miller hypomagnesemia in Type 2 Diabetes mellitus.(cjasn.asnjournal.org/content2/2/366.full.pdf)
15. Quamme GA: Dirks JH : The physiology of renal magnesium handling. Ren Physiol 9 : 257, 1986.
16. Rebsomen L, Pitel S, Boubred F, et al. C-peptide replacement improves weight gain and renal function in diabetic rats. Diabetes & Metabolism 2006;32(3):223-228[PubMed]
17. Sales CR: De Falima Campos pedrosa L : Magnesium and diabetes mellitus : Their relation clin. mut. 25: 554- 562, 2006.
18. Seeling M.S. and Heggtveit, A., Am. J. Clin. Nutr., 1974,27, 59-79.Nutritional aspects of Magnesium metabolism(www.ncbi.nlm.nih.gov/pmc/articles/PMC1272307
19. Sjoquist M, Hung W, Johansson B-L. Effects of C-peptide on renal function at the early stage of experimental diabetes. Kidney International. 1998:54(3): 758-764.[Pubmed]
20. Text book of Biochemistry, DM Vasudevan and Sreekumars, 3rd Ed. Page 99-102.
21. Tietz text book of clinical chemistry 2nd edition. By Carl A. Burtis, Edward R. Ashwood pages 1325-1329, 1910-1913, 1955-1956.
22. Walker G.M. Magnesium 1986: 59-23.
23. Zhou.Q. Olinescu RM, Kummerow FA : Influence of low Mg. concentration in the medium on the antioxidant system in culture human arterial endothelial cells. Magnes Res 12: 19-29, 1999.
24. Zhou.Q. Mahfouz M.M. and Kummerow, FA Magnes Res. 1994, 7, 23-30.
25. Schlessinger J. New roles for Src Kinases in control of cell survival and angiogenesis.Cell.2000;100:293-296[PubMed]
26. Tongyai.S, Rayssinguier.Y, Motta.C., Gueux.E, Maurois.P, and Heaton.W.Am.J.Physiol 1989, 257 C 270 – C276.
27. Dzurik. R, Stelikiova K, Sprustova V, Fetkovska N: The role of magnesium deficiency in insulin resistance : AN in vitro study / J Hypertens 9 (Suppl6]:S 311-S313, 99].
28. Quamme GA: Renal handling of magnesium. In : Massry and Glassock's text book of nephrology, 4th edited by Massry, SH, Glassock
29. Shakti Agarwal; Atul goel and Anju Jain .Role of C-peptide in identification of patients suspected of having Latent Autoimmune Diabetes in Adults(LADA) in North Indian Type2 DM population, IJPBS1(3),; July-Sep 2010
30. Vamsikrishna Chittimoju & Sujatha Pasula, Study of Serum Magnesium; HbA1c and microalbuminuria in Diabetic retinopathy; IJPBS 2013 July 4(3) 501-509
31. Shivakumar K: Profibrinogenic effects of Mg. deficiency in the cardiovascular system. Magnes Res 15 : 307-315, 2002.
32. Linda W.H.,Folsom A.R.,Neito J.,Mo J.P.Watson R.L.,Brancatti F.L.Serum and dietary Magnesium and the risk for Type 2 diabetes mellitus. The atherosclerosis risk in community study. Arch Intrn Med 1999;159:2151-9