Abnormal Cholesterol Uptake Rate Of Leucocytes Reflecting Metabolic Aberration In Non Insulin Dependent Diabetes Mellitus With lschem1c Heart Disease Patients

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ABSTRACT

Objectives-To *assess the lipid and lipoprotein abnormalities, oxidative stress, the effect of antioxidant defences to protect the beta cells from glucotoxicity and also to assess the uptake of cholesterol avidly by the normal lymphocyte cells, diabetic and diabetic with Ischemic heart diseases(IHD) cells.* ***Method-:*** *60 clinically diagnosed cases of type 2 diabetes mellitus with the age range 30-75 years were included in the study group. Out of which 30 diabetic patients with good glycemic control were included under Group I with age and sex matched healthy individuals served as controls and 30 diabetic patients with/without complications were included under Group II& Ill.* ***Results:*** *The fasting blood glucose were significantlyhigher in {p<.001) when compared with controls and parallel increase is seen in HbAlC in group I/&IIJ. Similar trend is observed in triglycerides and free fatty acids. Lipid peroxidation levels increases nearly five fold in diabetic patients with /HD.SOD, CAT activities are low while GPx level is raised with increased DNA strand breakage in group II.* ***Conclusion-*** *In conclusion, with progression of diabetes, i.e development of complications shows increase in fa sting blood glucose, cholesterol distribution in lipoprotein, lipid per oxidation and role of antioxidants.*

Keywords: *- Type2 Diabetes Mellitus, Glycated hemoglobin, Dyslipidemia, Cardiovascular*

Introduction

Non-insulin-dependent diabetes mellitus **(NIDDM)** constitutes about 95% of all the diabetic patients seen in Southern India.

The prevalence of ischemic heart disease is higher among patients with both diabetes and impaired glucose tolerance (IGT) compared to the general population [2-4]. Recent studies have shown that the overall prevalence of IHD is higher among migrant Asian Indians compared to Europeans [5, 6]. It is also ofinterest that IHD occurs at a younger age among migrant Indians in the UK [7]. There are few data on IHD in NIDDM patients from our country. The high global burden of IHD is highlighted by the world health report which estimates that 30.9% of all deaths in 1998 as well as 10.3% of the total disease related burden were attributed to cardiovascular disease (8). IHD could be diagnosed as chest pain arising from the heart, usually under the

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sternum due to an inadequate supply of oxygen to the heart muscle. The prevalence of IHD increased with age and duration of diabetes in both males and females and 40.1 % of those with over 20 years duration of diabetes had IHD. (9) The prevalence of all other diabetic complications like peripheral vascular disease, nephropathy and retinopathy was higher in the patients with IHD.

Materials and methods

The study group comprised of 60 consecutive NIDDM out patients at Royapettah hospital, Chennai. The diagnosis of diabetes and the classification as NIDDM were based on the WHO study group criteria [8]. A detailed clinical history was taken in all patients including the duration of diabetes mellitus, age at onset, previous history of angina/infarction and a history of smoking. All patients underwent a thorough clinical examination which included recording of height and weight, body mass index (BMI), blood pressure, palpation of peripheral pulses, auscultation for carotid or femoral bruit and elicitation of the knee and ankle jerks are vibration sense. The patients clinicallydiagnosed of type 2 diabetes mellitus with the age range 30-75 years were included in the study group. Out of which 30 diabetic patients with good glycemic control were included under Group I with age and sex matched healthy individuals served as controls and 30 diabetic patients with/without complications were included under Group ll. Casehistory, family history, height, weight,food and smoking habits were recordedusing standardized health profile (SH P)for all the patients and volunteers in thestudy. Blood was drawn by vein puncturefrom subjects after 12 hours over nightfasting. The fasting blood samples werecollected in an EDTA/heparin/ plain bulbvial for estimation. Plasma glucose, serumtriglycerides, total cholesterol, HDL cholesterolwere measured usingenzymatic kit methods. LDL-cholesteroland VLDL cholesterol were calculatedusing Friedewalds formulae (10).Superoxide dismutase (SOD) estimationwas based on the reaction between
superoxide radicals and 2-4 iodophenyl 3- 4 nitrophenol-5-phenyl tetrazolium chloride (11,12). Glutathione peroxidase (GPx) was measured by the method of Paglia and Valentine (13). lsolation of DNAfrom lymphocytes were carried out according to the method of I was a et al.(1996). Agarose gel is used extensivelyfor the separation of DNA restrictionfragments because of its back of molecular sieving and electrophoresis.

Statistical Analysis

All quantitative measurements are expressed as mean_ +standard deviation (S.D) for control and experimental values separately. Statically significant difference between the diabetics with/without complications and controls were arrived at using two tailed students't' test, followed by

analysis of variance - multiple range tests· student- - Newman –Keuls test with significnace

Results and Discussion

Table 1 shows the patient characteristics of NIDDM with ischemic Heart disease(IHD)indicates 80% were female and remaining 20% were male. 30% were affected due to their lifestyle, 50% due to stress and 20% due to strong family history at the time of' diagnosisSmoking and alcohol is one of the way to ischernic heart disease with NIODM (10%chewing - female. Dietary pattern for patients were 90% non veg and 10% veg. BMR level is raised according to their severity of complications.

Table-1: General data of the population investigated and their daRslflcatlon

Groups	Normal volunteers without any disease (GROUP I)	Diabetes mellitus without any complications (Group II)	Diabetes mellitus with complications (GROUP III)
Number	N=30	N=13	N=17
Duration of diabetes mellitus	-	10	5-10
Family history of diabetes mellitus	18%	85%	75%
Males	20%	60%	30%
Females	80%	40%	70%
BMI Kg/m^2	25	34.2	38.9
Dietary history and smoking	90%	95%	90%
Previous history and smoking	65%	85%	88%
Females (Betal chewing)	-	15%	12%
Drugs administered		Metformin glibenclamide	Atenolol,Nifidepine,Amylodepin

The fasting blood glucose, hemoglobin and glycemic control values arc presented intable 2. Fasting blood glucose were (statistically) significantly higher in NIDDM with IHD and increased in the severity of complications with mean value of 265±49.4 mg/ dl lnspite of their oral hypoglycaemic drug therapy. Parallel increase Is seen In glycosylated hemoglobin and marginal decreases in haemoglobin were observed

Table-2: Levels of glucose, Hemoglobin, HbA1C in normal and NIDDM patients with andwithout complications.

Groups	Classification	Glucose(mg/dl)	Haemoglobin(g/ dl)	Glycosylated Hb
Group I	Normal	107.0 ± 16.8	12.7± 1.7	7.1 ± 1.0

Group II	Diabetes withoutcomplication	261.8 ± 32.6a***	9.0 ± 1.5a***	11.1 ± 1.6a***
Group II	Diabetes with ischemic heart disease	265.9 ± 49.4a***b*	8.4 ± 0.6a***b*	12.1+ 2.2***b*

Statistically significant differences are denoted as follows:
a*** - significant at p<0.001 respectively when compared with controls (Group I)
b* - significant at p<0.001 respectively when compared with NIDDM without complications

Table-3: Lipoproteins in normal and N IDDM Patients with and wi thout complications

Groups	Classification	Glucose (mg/dl)	Haemoglobin (g/dl)	Glycosylated Hb(%)
Groups I	Normal	107.0±16.8	12.7±1.7	7.1±1.0
Groups II	Diabetes without complications	261.8±32.6a***	9.0±1.5a***	11.1±1.6a***
Groups III	Diabetes with ischemic heart diseases	265.9±49.4a***b*	8.4±0.6a***b*	12.1±2.2a***b*

Statistically significant differences are denoted as follows:
a*** - significant at p<0.001 respectively when compared with controls (Group I)
b***,b* - significant at p<0.001,p<0.05 respectively when compared with NIDDM withoutcomplications (group II)

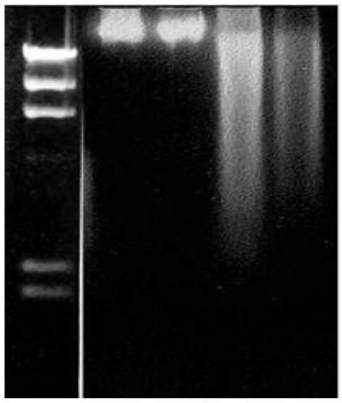
Table-4: Levels of ant oxidation in normal and NIDDM Patients with and without complications

The values are expressed in mean ± standard deviation

Groups	Classification	SOD (U/mg protein one unit correspond to 50% inhibitions of pyrogallol	Catalase (nmol of H2O2 decomposed/ Min /mg protein)	GPX if of glutathione utlised /min /mg protein
Groups I	Normal	3.5±0.3	4.4. ±0.4	5.4±1.2
Groups II	Diabetes mellitus	2.4±0.2a***	3.7±0.5a***	6.3±1.1a***
Groups III	Diabetes mellitus with ischemic heart diseases	2.1±0.2a***b*	3.2±0.3a***b***	8.2±2.3a***b***

Statistically significant differences are denoted as follows:
a*** - significant at p<0.001 respectively when compared with controls (Group l)

b* - significant at p<0.05 respectively when compared with NIDDM withoutComplications (Group II)



Lane 1& 6 : Normal Healthy Volunteer

Lane 2: NIDDM patients with complication

Lane 3,4 & 5 : NIDDM patients without complication

In the present investigation, DNA damage was studiedby isolating the DNA and subjecting it to agarose gelelectrophoresis. Lane1 and 6 shows migration ofnormal DNA whereas lane 2,3,4 and 5 showsabnormal migration of DNA because of the damage ofDNA molecules in patients of NIDDM with/withoutcomplications. From the gel, it was observed that DNAisolated from the diseased state showed markedoxidative damage to all four DNA bases. This wasparalleled by an increased DNA strand breakage in the diseased lymphocytes.The vascular and othercomplications of diabetes are suggested to involveoxidative damage resulting from the hyperglycemiaand/or hyperlipidemia(ll)

Conclusion

In conclusion, with progression of diabetes, i.e development of complications showsincrease in fasting blood glucose, cholesterol distribution in lipoprotein, lipid per oxidationand role of antioxidants. The studies made on lymphocyte uptake of cholesterol fromplasma is preliminary study, has revealed that in NIDDM with IHD , metabolic andregulatory changes have already taken place making the cells more susceptible tocholesterol accumulation possibly through increased number of scavenger receptorsand/or oxidised LDL, VLDL and HDL on the caveolae affecting cholesterol efflux. The needto study oxidative modifications in VLDL and its uptake by the peripheral cells have beenhighlighted.

References

1. Mohan V, Alberti KGMM. Diabetes in the Tropics. In: International Textbook of Diabetes Mellitus. Alberti kgmm Defronzo RA, Keen H, Zimmet p (eds.). John Wiley and Sons Ltd, Chichester 1991; 177-96.

2. Kannel WB, McGee DL. Diabetes and cardiovascular disease The Framingham Study. J Am Med Asso 1979; 24: 2035-8.

3._ Jarreft RJ,_ McC~rtney P, Keen H. The Bedford Survey: ten year mortality rates in newly diagnosed d1abet1cs, border line diabetes and normoglycaemic controls and risk indices forcoronary heart disease in borderline diabetics. Diabetologia 1982; 22 : 79-84.