

Abnormal Antioxidant Status in Impaired Glucose Tolerance and Non-insulin-dependent Diabetes Mellitus

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A total of 105 subjects with impaired glucose tolerance were classified into two groups, 51 subjects with plasma glucose $> 11.1 \text{ mmol l}^{-1}$ in one of the blood samplings during OGTT, but at 2 h being less than $< 11.1 \text{ mmol l}^{-1}$ were classified as early hyperglycaemics. Fifty-four cases were classified as true IGT, with fasting plasma glucose $< 7.8 \text{ mmol l}^{-1}$ and post plasma glucose level between 7.8 and 11.1 mmol l^{-1} . Age and sex matched groups of normals (healthy adults) and NIDDM cases without symptomatic secondary complications were also included in the study. Lipid peroxidation (LPO) product in plasma, erythrocyte, and erythrocyte cell membrane were found to be significantly elevated ($p < 0.001$) in IGT, early hyperglycaemia and diabetes mellitus while glycosylated haemoglobin was also higher. Antioxidant enzymes superoxide dismutase and catalase were significantly lower in red blood cells obtained from IGT and early hyperglycaemic groups. They were closer to the levels showed in NIDDM confirming that antioxidant deficiency is already present in subjects classified as impaired glucose tolerant. Among the antioxidant scavengers, reduced glutathione (GSH) and ascorbic acid are reduced by 15 % and 20 % in IGT and NIDDM, respectively. We conclude that antioxidant status is poor in both IGT and NIDDM, suggesting an overlap of frank diabetic state in those classified as IGT. It is possible that antioxidant therapy might retard progression from IGT to NIDDM.

KEY WORDS Impaired glucose tolerance Early hyperglycaemia NIDDM Lipid peroxidation Membrane LPO Antioxidant enzymes and scavengers

Introduction

Impaired glucose tolerance (IGT) is a diagnostic category coined by the National Diabetes Data Group (NDDG)¹ and adopted by the World Health Organization (WHO).² Individuals included in this category are those whose fasting plasma glucose (FPG) concentration is less than 7.8 mmol l^{-1} and whose 2 h plasma glucose concentration after a 75 g oral glucose tolerance test (OGTT) is between normal and diabetic values (7.8 and 11.1 mmol l^{-1}).

IGT as a risk factor for non-insulin-dependent (Type 2) diabetes mellitus (NIDDM) has received much attention.³ Abdominal obesity, family history of diabetes, glucose and serum insulin levels both fasting and after a glucose load, are all also risk factors for NIDDM.⁴

Reactive oxygen species (ROS) are increasingly formed in diabetes mellitus (DM) by the auto-oxidation of glucose and glycosylated proteins.⁵ Baynes⁶ postulated a general pathway involving a sequence of events by which oxidative stress leads to tissue damage in diabetes mellitus. According to this, increased formation of ROS and free radicals and their incomplete scavenging lead to peroxidation of cell membrane lipids, inactivation of proteins and fragmentation, crosslinking of DNA and tissue degeneration. Increased lipid peroxidation (LPO) and reduced levels of antioxidants, especially superoxide dismutase (SOD), catalase (CAT), reduced glutathione

(GSH) and ascorbic acid (vitamin C), are reported in NIDDM.⁷

The investigation presented here is on the assessment of antioxidant status and lipid peroxidation, in subjects with IGT. This is compared with healthy subjects and with patients with established NIDDM.

Patients and Methods

The following five groups of subjects were studied:

1. IGT was diagnosed according to the WHO criteria, i.e. fasting plasma glucose $< 7.8 \text{ mmol l}^{-1}$ and 2 h glucose (after 75 g oral glucose load) between 7.8 and 11.1 mmol l^{-1} . Among the 113 subjects who fell into this category, 54 showed plasma glucose concentrations $< 11.1 \text{ mmol l}^{-1}$ in the blood sampling at 30, 60, and 90 min also. They are classed as true IGT.
2. Fifty-one IGT subjects had fasting plasma glucose $< 7.8 \text{ mmol l}^{-1}$, while one of the 3 samples (30, 60 or 90 min after glucose load) was above 11.1 mmol l^{-1} , although at 2 h plasma glucose had dropped below 11.1 mmol l^{-1} . This group is classified as early hyperglycaemia.
3. Seventeen subjects showed fasting plasma glucose $< 7.8 \text{ mmol l}^{-1}$ and 2 h post glucose, plasma level

> 11.1 mmol l⁻¹. Their HbA_{1c} was in the range of 5.2 to 8.4 %. Eight out of the 17 cases had NIDDM diagnosed at the time of the study. This group of 17 are classified as early DM.

4. Sixty-seven cases of NIDDM without any secondary complications attending the M.V. Diabetes Specialities Centre, Madras-14 were studied. Their blood glucose at fasting and 2 h post glucose load were > 7.8 and 11.1 mmol l⁻¹.
5. Ninety subjects with normal pattern during oral glucose tolerance test (OGTT) (with 75 g glucose load) were included as healthy controls. They had come to the centre for diabetes check up, either because of family history or as a precautionary measure.

All subjects were also screened for other complications by routine clinical examination including chest X-ray, ECG, Doppler examination for peripheral vessel disease, X-rays to detect pancreatic calculi, ophthalmic examination and laboratory tests including blood haemoglobin, plasma albumin, serum transaminases, and microalbuminuria. A detailed case history which included physical measurements, duration, and family history of diabetes and food habits was obtained from each individual.

OGTT was performed with 75 g glucose load, and blood glucose assay at 0, 30, 60, 90, and 120 min by the glucose oxidase method (Corning Plus Autoanalyser). An aliquot of fasting blood sample was collected with EDTA (1 mg ml⁻¹) as anticoagulant. Glycosylated haemoglobin (HbA_{1c}) was measured according to Wang and Yang⁸ in the haemolysate. The erythrocyte membrane was isolated according to the method of Dodge *et al.*⁹ and modified by Shanmugasundaram *et al.*¹⁰ Lipid peroxidation was assayed as thiobarbituric acid reactive species (TBARS) and expressed in terms of malondialdehyde (MDA) according to the method of Yagi *et al.*¹¹ in the plasma and by the method of Cynamon *et al.*¹² in the erythrocyte and erythrocyte membrane.

The enzymatic and non-enzymatic antioxidants were also assayed. Glutathione peroxidase (GPx) and superoxide dismutase (SOD) were assayed in the haemolysate by the methods of Rotruck *et al.*¹³ and Misra and Fridovich,¹¹ respectively. Membrane bound catalase was assayed according to Sinha.¹⁵ Vitamin A¹⁶ and vitamin E¹⁷ were estimated in the plasma, while vitamin C¹⁸ and reduced glutathione¹⁹ were estimated in the whole blood.

Statistical Analysis

Statistically significant differences between the values of IGT, early hyperglycaemia, and NIDDM when compared to controls were obtained using Student's *t*-test. The values are expressed as mean ±SD for the five different groups separately.

Quality control of laboratory analysis was maintained over the period studied and technical error was arrived

at by the following formula and as described by earlier workers²⁰ using 25 samples for each test.

$$\sqrt{\frac{\sum d^2}{2N}} \times \frac{100}{\text{Total sample mean}}$$

where *d* = difference between duplicate values for the same sample

N = number of samples analysed.

The technical error was found to be 1.6 % SOD, 1.06 % CAT, 0.81 % GPx, 0.2 % GSH, 1.2 % vitamin A, 1.3 % vitamin E, 1.0 % vitamin C, 0.55 % plasma LPO, 1.04 % erythrocyte LPO, and 0.85 % erythrocyte membrane LPO.

Results

Table 1 shows the clinical and anthropometric characteristics of the study group classified according to the glucose tolerance status. Around 80 % of the diabetic population and the IGT and early hyperglycaemia subjects had a positive family history of diabetes. The mean value of body mass index (BMI) in subjects with diabetes was comparable to the control population. HbA_{1c} (6.2 ± 1.1 %) was significantly higher in those classified as early hyperglycaemia.

Lipid peroxidation in plasma, erythrocytes, and erythrocyte membrane is shown in Table 2. A statistically significant increase in lipid peroxidation was seen both in IGT and in early hyperglycaemia when compared to the healthy controls.

From Table 3, it can be seen that the antioxidant defences are weak in IGT. The antioxidant scavengers vitamins E and C and reduced glutathione (GSH) were significantly lower in IGT when compared to healthy controls. SOD and CAT are also markedly lowered. The antiperoxidative enzyme GPx was significantly higher (nearly 50 %) in those with impaired glucose tolerance, early hyperglycaemia, and NIDDM, when compared to the healthy controls.

Discussion

While the criteria for classifying IGT has two parameters, namely the fasting plasma glucose level of < 7.8 mmol l⁻¹ and a 2 h glucose level between 7.8 and 11.1 mmol l⁻¹, a significant number of subjects have their plasma glucose rise above 11.1 mmol l⁻¹ during the early phase of the OGTT (i.e. within 90 min) and their hyperglycaemic condition is acknowledged in our classification into early hyperglycaemia. Both IGT and early hyperglycaemia groups show statistically significant rise in HbA_{1c}, suggesting that with the present criteria for IGT classification, subjects with diabetic pathological changes may also be labelled as having IGT, and hypoglycaemic therapy delayed or denied to them at the early stages. In other centres also²¹ subjects with IGT had higher than normal levels of glycated haemoglobin as in our study.

Table 1. General data of the population studied

Subjects	Healthy controls (n = 90)	Impaired glucose tolerance		Early DM (n = 17)	NIDDM without complications (n = 67)
		IGT (n = 54)	Early hyperglycaemia (n = 51)		
Duration of DM (range)	-	-	-	0-8 months	7 months-5 years)
Age (yr)	47 ± 6	45 ± 9	44 ± 10	45 ± 8	44 ± 9
Males (%)	68	62	61	60	75
Family history of DM ^b	30	80	76	92	83
BMI (kg m ⁻²)	24 ± 5.0	26 ± 4.0	26 ± 3.0	25 ± 2.0	23 ± 8.0
Fasting blood glucose (mmol l ⁻¹)	4.9 ± 0.5	5.49 ± 0.5 ^a	6.47 ± 3.3 ^a	8.45 ± 2.7 ^a	9.5 ± 3.7 ^a
HbA _{1c} (%)	4.9 ± 0.5	6.2 ± 1.1 ^a	6.8 ± 1.0 ^a	8.6 ± 2.0 ^a	11.2 ± 1.5 ^a

IGT: fasting blood glucose < 7.8 mmol l⁻¹, postprandial 7.8-11.1 mmol l⁻¹.

Early hyperglycaemia: fasting blood glucose < 7.8 mmol l⁻¹, post prandial > 11.1 mmol l⁻¹

Statistically significant alterations from controls is expressed as ^ap < 0.001.

^bAt least one parent with DM.

Table 2. Lipid peroxidation in terms of MDA in the plasma, erythrocytes, and erythrocyte membrane in diabetes mellitus, impaired glucose tolerance, and healthy controls

Parameters	Healthy controls (n = 90)	Impaired glucose tolerance		Early DM (n = 17)	NIDDM without complications (n = 67)
		IGT (n = 54)	Early hyperglycaemia (n = 51)		
Plasma MDA (nmol dl ⁻¹)	190 ± 20	201 ± 40 ^a	223 ± 48 ^a	280 ± 25 ^a	311 ± 45 ^a
Erythrocyte MDA (nmol MDA 10 ⁻¹² cells)	13.0 ± 1.5	17.7 ± 5.8 ^a	20.1 ± 5.9 ^a	27.0 ± 5.0 ^a	35.2 ± 2.0 ^a
Erythrocyte membrane MDA (nmol MDA 10 ⁻¹² cells)	261 ± 44	317 ± 102 ^a	443 ± 117 ^a	490 ± 104 ^a	673 ± 119 ^a

Statistically significant alterations from controls is expressed as ^ap < 0.001.

Table 3. Antiperoxidative enzyme status and antioxidant scavengers in diabetes mellitus, impaired glucose tolerance and healthy controls.

Parameters	Healthy controls (n = 90)	Impaired glucose tolerance		Early DM (n = 17)	NIDDM without complications (n = 67)
		IGT (n = 54)	Early hyperglycaemia (n = 51) _s		
Glutathione peroxidase (µg GSH liberated/min/mg Hb)	5.9 ± 0.6	7.2 ± 1.2 ^a	7.3 ± 1.1 ^a	7.2 ± 0.3 ^a	7.2 ± 0.3 ^a
Superoxide dismutase (IU mg Hb ⁻¹)	3.4 ± 0.4	2.9 ± 0.4 ^a	2.6 ± 0.3 ^a	2.5 ± 0.4 ^a	2.6 ± 0.3 ^a
Catalase (µmol H ₂ O ₂ consumed/min/ 10 ¹² cells)	4.4 ± 0.5	4.1 ± 0.7 ^a	3.9 ± 0.8 ^a	3.9 ± 0.6 ^a	3.8 ± 0.6 ^a
Vitamin A (µmol l ⁻¹)	2.21 ± 0.19	2.19 ± 0.13	2.14 ± 0.19	2.13 ± 0.17	2.18 ± 0.16
Vitamin E (µmol l ⁻¹)	25.1 ± 1.6	22.4 ± 2.3 ^a	21.9 ± 1.9 ^a	21.4 ± 2.1 ^a	20.0 ± 2.3 ^a
Vitamin C (µmol l ⁻¹)	60.9 ± 7.3	48.8 ± 12.7 ^a	47.6 ± 12.1 ^a	44.7 ± 8.0 ^a	44.3 ± 5.5 ^a
GSH (µmol l ⁻¹)	53.4 ± 6.0	45.3 ± 7.1 ^a	45.0 ± 6.2 ^a	45.1 ± 4.5 ^a	45.6 ± 6.5 ^a

Statistically significant alterations from controls is expressed as ^ap < 0.001.

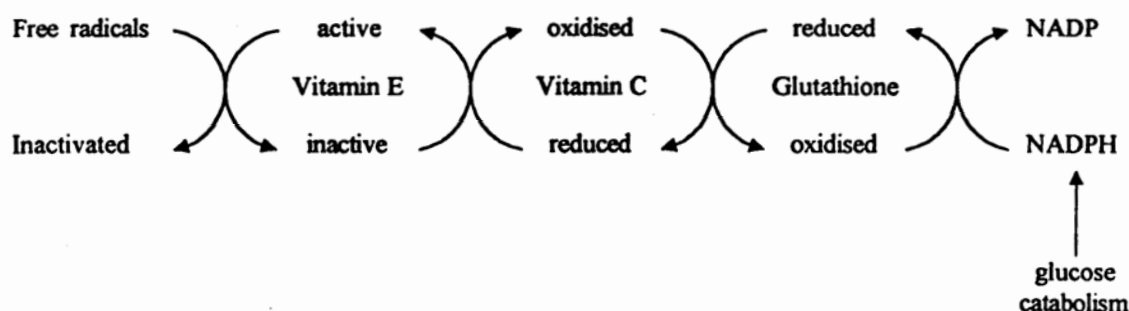


Figure 1. Interrelationship between antioxidant scavengers according to Niki (1987)

Kadowaki²² observed that the initial degree of hyperglycaemia is an important index predictive of subsequent worsening to diabetes. Marshall *et al.*²³ observed that subjects with IGT have an increased risk of developing NIDDM.

We found that the lipid peroxidation products in the plasma, erythrocyte, and cell membrane were significantly elevated in IGT. Lipid peroxidation was 10% higher in plasma and 20% higher in red cell membrane obtained from IGT subjects when compared to healthy controls.

The elevated levels of lipid peroxidation in plasma, cells, and cell membrane in our subjects with IGT and NIDDM were accompanied by diminished antioxidants and this increased with increasing glucose intolerance, in that the abnormalities were greater in those with frank diabetes than in those in early hyperglycaemia and impaired glucose tolerance. In contrast to the effect of glucose intolerance on the antioxidant enzymes SOD and catalase, we found that the peroxide inactivating enzyme glutathione peroxidase (GPx) was raised in IGT, early hyperglycaemia and also in symptomatic NIDDM. However, GPx requires glutathione in its reduced form (GSH) as a cofactor to enable it to inactivate peroxide and function as an effective antioxidant. Our data show significantly lower levels of GSH in IGT, early hyperglycaemia, and NIDDM. Thus, the effective peroxide detoxification in the cell appears to be impaired and this may contribute to antioxidant deficiency.

Normally the body has an abundant supply of 'antioxidants' that delay or inhibit oxidation and neutralize the reactive oxygen species. Glutathione is endogenously synthesized predominantly in the liver²⁴ and in other tissue cells. It is the first line of defence against prooxidant stress.²⁵ It exists in the oxidised (GSSG) and reduced (GSH) forms which are interconvertible. GSH in turn keeps up the reduced forms of vitamins C and E, which participate in neutralizing the free radicals as and when they are formed.²⁶ (Figure 1). Thus, GSH is able to spare vitamins C and E in the cell in combating free radicals at the cytoplasmic and membrane level, respectively.

In IGT the antioxidant scavengers are already low, and this may account for the higher lipid peroxidation

products seen. The observation presented is the first demonstration that in IGT altered antioxidant status is already developed and may contribute to tissue damage resulting in progression to NIDDM. The subjects with IGT are already presenting symptoms of metabolic derangements due to hyperglycaemia like elevated HbA_{1c}, increased lipid peroxidation, and mild antioxidant depletion. A rethinking on the criteria for NIDDM may not be inappropriate. It would be interesting to study the effect of antioxidant therapy in subjects with IGT, and follow-up their glucose tolerance in the next couple of years to check whether antioxidant therapy could check the development of symptomatic diabetes mellitus.

Acknowledgements

We express our gratitude to R Mohan of M.V. Diabetes Specialities Centre, Madras, India for providing the required blood samples and the clinical picture, and to R.D. Birla Smarak Kosh for financial aid provided to A. Parthiban during the period of the study.

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