CHANGES IN ERYTHROCYTE GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) AND REDUCED GLUTATHIONE (GSH) ACTIVITIES IN THE DEVELOPMENT OF SENILE AND DIABETIC CATARACTS

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Abstract. Oxidative and osmotic stress have been implicated in the pathogenesis of cataracts. Reactive oxygen intermediates (ROI) mediate peroxidation of membrane lipids and cause irreversible damage to lens proteins. The purpose of this study was to assess the changes in erythrocyte glucose- 6-phosphate dehydrogenase enzyme (G6PD) and reduced glutathione (GSH) levels in the development of senile and diabetic cataracts. The activity of erythrocyte G6PD and the concentration of GSH were measured to assess changes in oxidation-reduction status. The oxidation-reduction status of 26 non-diabetic non-cataract (control) subjects were compared with 24 diabetic non-cataract, 30 diabetic cataract and 28 non-diabetic cataract subjects. The results revealed that the GSH and G6PD levels of the subjects with senile cataracts were significantly lower than the subjects without cataracts. The present study reveals the risk of developing senile cataracts is associated with decreased levels of erythrocyte G6PD and GSH. In the formation of diabetic cataracts an adequate supply of NADPH (G6PD activity) is essential to produce osmotically active sorbitol in the lens.

INTRODUCTION

Cataracts are one of the leading causes of blindness in the world today (Bunce *et al*, 1990). The majority of blindness can be attributed to cataracts and more than twenty million people worldwide are affected (Taylor and Nowell, l999). The health of the lens depends greatly on the production of reducing power which helps to keep the proteins in their reduced state. Cataracts develop due to the concerted action of a) accumulation of sorbitol within the lens, and b) the oxidized state of the lens proteins (crystallins) in diabetic individuals (osmotic cataracts) as well as in non-diabetic individuals (senile cataracts). It is believed that osmotic and oxidative stress are involved in the pathogenesis of cataracts (Halliwell, 1997).

Oxidative stress may result when the cellular antioxidant defense mechanisms are unable to keep pace with the detoxification of reactive oxygen intermediates (ROI). ROI has been implicated in cataractogenesis (Davies, 1995), and ROI mediated peroxidation of membrane lipids can cause extensive damage to proteins leading to irreversible deleterious effects (Micelli-Ferrari *et al*, 1996). Studies of the antioxidant enzyme status in the blood and in the lens tissues indicate that erythrocyte antioxidant enzymes levels are lower in people with senile cataracts than in those with osmotic cataracts (Ozmen *et al*, 2002; Chandrasena *et al*, 2006). Therefore,

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oxidative mechanisms are believed to play a vital role in the pathogenesis of senile cataracts.

Osmotic stress develops due to accumulation of glucose, which is then converted to sorbitol, which can lead to the development of osmotic cataracts in diabetic individuals (Fig 1). The primary purpose of the hexose monophosphate shunt (HMP) is to generate reducing power in the cytoplasm in the form of reduced nicotinamide adenine dineucleotide phosphate (NADPH). This conversion of glucose to sorbitol needs NADPH and hence the need for proper functioning of the HMP pathway.

The key regulatory enzyme of HMP is G6PD. NADPH is also needed in the formation of GSH, which maintains cell membrane proteins in stable reduced form (Marjorie, 2003). Thus any reduction in the activity of this enzyme could lead to instability of the tissues that depend on a continuous supply of NADPH. Primary amongst these tissues are erythrocytes, but it is also present in other tissues, such as the lens, kidneys, adrenals, liver and platelets (Babizhayer *et al*, 1992).

It has been reported that persons with G6PD deficiency are at high risk for developing cataracts whilst uncontrolled diabetics are at a higher risk (Babizhayer *et al*, 1992; Marjorie, 2003). The enzyme aldose reductase which produces intermediate sorbitol is a NADPH requiring enzyme with a high Km for glucose (Crabble and Goode, 1998), thus elevated intracellular glucose concentrations and an adequate supply of NADPH leads to a

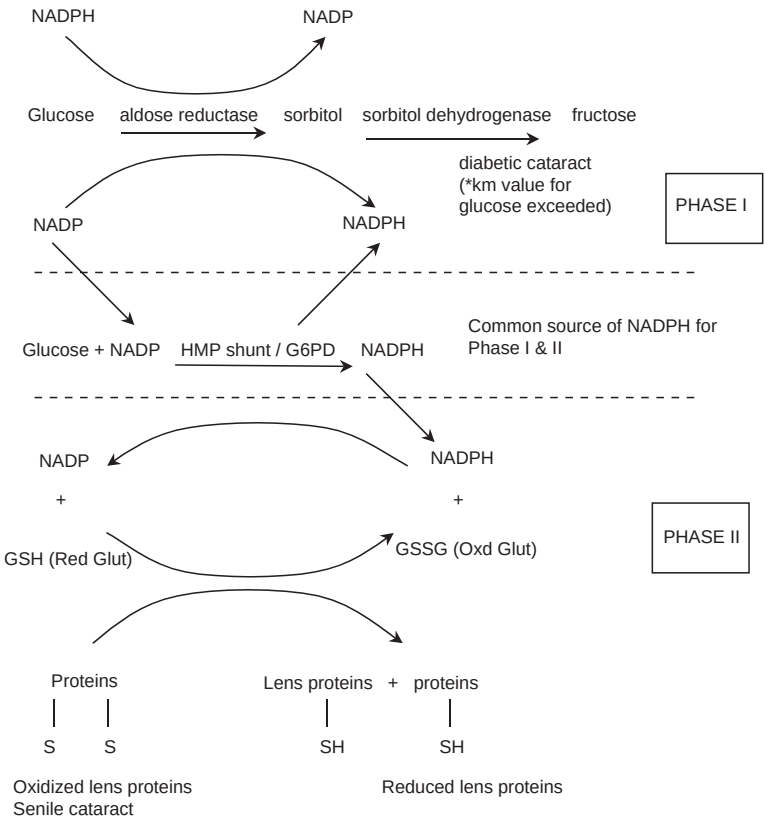

Fig 1–HMP (G6PD) and its effects on cataract formation in diabetics and non-diabetics.

significant increase in the amount of sorbitol. Sorbitol, unlike glucose, cannot pass efficiently through cell membranes and therefore remains trapped inside the cells, increasing osmolality, resulting in swollen cells due to water retention. This pathological alteration (opacity) in the lens tissue leads to cataract formation and is exacerbated in certain tissues low or deficient in sorbitol dehydrogenase enzyme. Therefore, diabetics with normal G6PD levels and with sufficient amounts of NADPH, are at greater risk for developing cataracts than diabetics with reduced G6PD levels (Babizhayer, 1992).

The purpose of this study was to assess the oxidation reduction status of erythrocytes in diabetic and healthy subjects and the relationship between the oxidation-reduction status in the formation of cataracts in diabetic and healthy subjects in Sri Lanka.

MATERIALS AND METHODS

Study population

A total of 108 subjects, including 54 diabetics on oral hypoglycemic drugs with and without cataracts, 28 non-diabetic (senile) cataract patients in the age 50-80 years old who were residents of North Colombo in western Sri Lanka were studied and compared with 26 healthy controls from the same area. Written informed consent was obtained from all subjects at the time of recruitment into the study.

Ethical clearance for the study was obtained from the Ethics Committee of the Faculty of Medicine, University of Kelaniya, Sri Lanka.

Blood sampling and data collection

A data sheet on the past medical history and other relevant information was completed for each subject. A complete ophthalmological examination, including a silt lamp examination, was done by an ophthalmologist. Cataracts were defined as lens opacities visible on axial or oblique illumination when viewed with a slit lamp or by direct ophthalmoscopy. Blood samples were obtained from all subjects in the non-fasting state at the time of attending the clinics, or in the case of home visits random blood samples were obtained. Venipuncture was done in the sitting position and 5 ml of blood was obtained. Blood was placed into EDTA bottles and brought to the laboratory without delay and analyzed within 3 hours of collection.

Laboratory analysis

The activity of G6PD was assayed as described by Meloni *et al* (1990) using commercially available reagent kits (Randox Laboratories, Crumlin, Antrim, UK. In this method, the enzyme activity was determined by measurement of the rate of change of absorbance at 340 nm due to the reduction of $NADP^+$. The concentration of GSH was measured as described by Beutler (1975). All analyses were performed at the Department of Biochemistry and Clinical Chemistry, Faculty of Medicine, University of Kelaniya with quality control procedures to ensure the levels of precision and accuracy required. The results were analyzed using the Student's *t*-test and EPI info 6 software.

RESULTS

No significant difference ($p>0.05$) was observed in G6PD levels in subjects with or without cataracts. However, the GSH levels of the subjects with cataracts were significantly ($p<0.05$) lower than the subjects without cataracts (Table 1).

The G6PD and GSH levels in those with diabetic cataracts, diabetic without cataracts, non-diabetic cataracts and healthy subjects are given in Table 2. Although the levels observed for G6PD and GSH in the diabetic subjects were lower than the control subjects, the values were not significantly different. How-

Table 1
G6PD and GSH levels in subjects with cataracts and without cataracts.

	With cataracts (n = 58)	Without cataracts (n = 50)	Statistical significance
G6PD	138.90 ± 25.52	147.60 ± 51.12	NS
GSH	70.47 ± 20.40	83.43 ± 23.71	< 0.05

Mean ± SD (n) values for G6PD in MU/10 ^{9} packed RBC, RBC GSH in mg/l packed RBC are given in the table.

Table 2
G6PD and GSH levels of four different groups of subjects.

	Non-diabetic without cataract (normal) (n = 26)	Diabetic without cataract (n = 24)	Diabetic with cataract (n = 30)	Non-diabetic with senile cataract (n = 28)
G6PD	151.7 ± 27.3	142.1 ± 27	147.4 ± 29.3	134.4 ± 19.8[a]
GSH	79.7 ± 26	87.4 ± 22	81.4 ± 19	57.6 ± 13.6[b]

G6PD in MU/10^9 packed RBC and GSH in mg/l packed RBC
[a]Significantly lower ($p<0.05$) than the other three groups
[b]Two groups were significantly different at $p<0.05$

ever, G6PD and GSH levels were significantly ($p<0.05$) lower in subjects with senile cataracts when compared with the other three groups.

The development of cataracts in diabetics is due to factors such as accumulation of glucose and its conversion to sorbitol resulting in an osmotic stress.

Although not significant, lower levels of G6PD were observed in diabetics without cataracts than healthy subjects. However, they had higher GSH levels than normal healthy subjects.

Higher levels of G6PD and lower GSH levels in diabetic subjects with cataracts than in diabetic subjects without cataracts suggests a significant role of increased GSH in preventing or delaying cataract formation, especially in diabetic non-cataract subjects.

DISCUSSION

Oxidative mechanisms play an important role in the pathogenesis of cataracts. Research regarding antioxidants has focused mostly on either senile or diabetic cataract patients. Risk factors for development of cataracts in non-diabetics without cataracts, non-diabetics with cataracts and those with senile cataract have not been carried out in a single study. Our findings add more information to the literature regarding the development of cataracts.

The biochemical pathways leading to the formation of cataracts in diabetics and non-diabetic subjects are depicted in Fig 1. These biochemical events can be separated into Phase I and Phase II. The Phase 1 predominates in uncontrolled diabetes where the km^3 value of aldose reductase is exceeded by high glucose levels, leading to high levels of sorbitol utilizing NADPH, which is produced by the HMP shunt via G6PD. Any deficiency of G6PD or NADPH would therefore slow down accumulation of sorbitol and delay cataract formation. If there were adequate NADPH this would augment the formation of diabetic cataracts.

However, in such patients if there is a high demand on the reduced GSH levels due to increased oxidative stress, it would create a higher demand on the common NADPH source. Therefore the concerted action of Phases I and II will predispose these diabetic patients to a higher risk for cataract formation.

In the case of non-diabetic patients, cataract formation may be explained mainly by events in Phase II, in a non-diabetic senile cataract high oxidative stress could be due to increased ROI production or UV radiation which could impose a high demand on reduced GSH. If in such subjects this capacity to maintain adequatly reduced GSH levels can be reduced by inadequate NADPH levels (low G6PD) they could be predisposed to cataract formation. If such subjects have low G6PD (reduced levels of NADPH) they may be predisposed to forming cataracts. Unlike the previous case where there is no involvement of Phase I and the events are restricted to Phase II, the common denominator is the supply of NADPH for GSH. The results of our study are explained on the basis of this hypothesis.

The data were analyzed to examine the relationship between the presence of cataracts and the levels of GSH and G6PD irrespective of diabetes. The results reveal that all subjects irrespective of the nature of the cataract, were associated with lower levels of G6PD, though the relationship was not significant (Table 1), when compared to subjects without cataracts. A higher prevalence of cataracts in patients with G6PD deficiency was reported by Meloni *et al* (1990) in southern Sardinian men age 40-50 years old (Meloni *et al*, 1990). These findings are also in agreement with Zinkham (1961) who found cataractogenesis was associated with G6PD deficiency (Zinkham, 1961).

The GSH levels of all subjects with cataracts were significantly lower ($p<0.05$) than those of non-cataract subjects, which is in agreement with previous studies which showed lenses with cataracts had significantly low levels of erythrocyte GSH (Green, 1995). Therefore, with high oxidative stress there will be an increased drain on the reduced GSH pool leading to increase replenishment of GSH via NADPH from G6PD. However, if the drain on the NADPH is higher due to diabetes mellitus, these subjects will have a higher risk of developing cataracts. This further confirms our findings that diabetics with cataracts have lower GSH compared to those without cataracts. With senile cataracts, the decline in G6PD activity may be a consequence of increased exposure to environmental factors, such as ROI or UV radiation and may not be able to keep up the oxidative status. The high content of GSH in the lens is believed to protect thiols in structural proteins and enzymes keeping them in the reduced state for proper biological functioning, and reduced GSH levels were more likely to be associated with senile cataracts than osmotic cataracts, which is in agreement with our findings (Jacques *et al*, 1998).

The highest level of GSH among the four groups of subjects was found in the non-cataract group, which would have prevented the development of cataract in these subjects. This prevention must have been supported by adequate levels of G6PD resulting in adequate supplies of NADPH preventing cataract formation (Babizhayew, 1996).

Significantly low levels of reduced erythrocyte glutathione were also reported in subjects with diabetic and senile cataracts (Ganea and Harding, 2006). Previous work on the erythrocyte antioxidant status in patients with and without cataracts demonstrated a reduction in erythrocyte catalase, glutathione peroxidase and superoxide dismutase enzymes levels in patients with senile cataracts to a greater extent than osmotic cataracts (Donma *et al*, 2002; Chandrasena *et al,* 2006). In such instances events in Phase II denominated than those in Phase I (Fig 1).

Green (1995) hypothesized that cataract

formation may occur as a consequence of degradation of enzymes that normally metabolize and detoxify hydrogen peroxide and other free radicals allowing hydrogen peroxides to induce irreversible deleterious effects on different eye tissues. This data also reported reduced GSH levels (Table 2) indicating reduced capacity for detoxification in subjects with senile cataracts (Wan *et al,* 2006; Pinna *et al,* 2007).

In summary, this data confirms that a reduced oxidation reduction status (reduced levels of G6PD and GSH) of the erythrocytes is associated with the development of senile cataracts, and development of cataracts in diabetic subjects is dependent on an adequate supply of NADPH (G6PD activity) for the conversion of glucose to sorbitol.

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