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iochemical examination of red cells plays an important role both in clinical
diagnosis and in the understanding of fundamental biochemical and genetic
mechanisms. This is because of the relative homogeneity of erythrocytes
suspension and the convenience with which they are obtained. The mechanisms
for the development of microvascular complications in diabetic retinopathy was
poorly understood and there is lack of evidence for the role of HMP shunt enzymes
and antioxidant levels.

In view of this, the present study was carried out to know the relation
between hyperglycaemia, lipid peroxidation, enzymes of hexose monophosphate
shunt and antioxidant status with diabetic retinopathy. Blood sugar was assessed
by measuring the levels of HbA1C in order to know the hyperglycaemic status.
Lipid peroxidation was measured in erythrocyte and plasma as MDA and DC
respectively. The enzymes of hexose monophosphate shunt studied were G-6-PD,
6-PGD, TK and TA of red blood cells. The enzymes of glutathione system and the
main biochemical parameters for antioxidant examined were GR, GSH, GST,
GSH-Px, catalase and SOD.

It is learnt from Table 4.1 that out of the 58 cases of diabetics with
retinopathy studied, 34 (58.6%) were BDR and 24 (41.4%) were PPDR. The
average duration of diabetes to develop BDR in uncomplicated diabetes were
11.35 years and PPDR were 16.45 years (Table 4.1). It is also evident from Table 4.1 that in diabetics without retinopathy, BDR will develop only after a minimum of 6 years duration and PPDR only after 12 years duration. Table 4.2 also shows that after 15 years of duration of diabetics, the progress to PPDR increases.

The above deteriorations may be due to the failure of control of glycaemic level. So estimation of HbA1C in diabetic retinopathy is an index for the metabolic control. HbA1C in the present study (Table 4.3) showed that there was a significant difference in HbA1C in diabetics with or without retinopathy as compared to normal. Inter-group comparison also pointed out that in BDR and PPDR, HbA1C level was significantly higher than DM. When the concentration of HbA1C reached above 12% in diabetics with or without retinopathy, 68% was PPDR, 23% was BDR and 9% was DM (Table 4.4). If blood sugar was not controlled in diabetic mellitus, it was likely to get affected by the retina resulting in the formation of BDR and PPDR as evidenced from the present finding (Table 4.3). Similar findings were also obtained by Thornalley et al. (1996) that a significant positive correlation between HbA1C and retinopathy.

Brownlee et al. (1988) reported that high levels of glucose leads to pathological alternation in retina, glomerulii and coronary vessels. Brinchmann et al. (1992) reported that by long term control of blood glucose level, the progress of retinopathy can be retarded, but Lauritzen et al. (1985) failed to demonstrate it. HbA1C has a higher affinity for oxygen which cause slow release of oxygen to tissue. Many post-transitional events occur non-enzymatically in this disease processes (Harding, 1985). Its action in diabetic retinopathy is showed in Figure 5.1.
Many biological metabolites react with oxygen to form intermediates which are more reactive than the parent molecules. These intermediates include organic free radicals, superoxide, hydroxyl and hydroperoxyl radicals which can induce oxidation of biological materials, including thiol oxidation, lipid peroxidation and enzyme inactivation. Generation of oxygen radical occurs by hyperglycaemia (Wolff et al., 1991), which later leads to the microvascular complications such as retinopathy (Jones et al., 1986; Kakkar et al., 1995).
It is clear from the lipid peroxidation studies that in diabetic with or without retinopathy DC and MDA are highly significant than normal controls. Inter-group comparison shows a significant difference between diabetic with or without retinopathy. Similar observation were also reported by Tomiyama et al. (1976), Jones et al. (1986) and Bambolkar and Sainani (1995) that lipid peroxidation is increased in diabetic retinopathy. PPDR and BDR also points to the fact that significant differences in the levels of MDA and DC.

Diabetic retinopathy is accompanied by endothelial proliferation which is believed to be the result of poor long term glycaemic control and accumulation of glycosylation end-products (Tesfamariam and Cohen, 1992). It is clear from these observation that the formation of diabetic retinopathy may be due to the increase in free radicals by hyperglycaemia, lipid peroxidation and the end-product of glycosylation.

The increase in MDA and DC in diabetic retinopathy may be due to the decreased activity of antioxidants in diabetic retinopathy. Earlier, Jennings et al. (1987) and Jiang et al., (1996) also showed that antioxidants are reduced in retinopathic cases. The activity of glutathione system-related antioxidant is mainly depends on HMP shunt for energy. Fujii et al. (1984) and Sundaram et al. (1996) reported that GSH, GR, GSH-Px and GST take part in the defence against different form of free radical that are formed during oxidative stress in diabetes especially in ocular tissues. NADPH is a cofactor for GR which convert GSSG to GSH, decreased activity of which may affect the NADPH/NADP ratio. In the present study, there is a significant decrease in the activity of GR in diabetic with or without retinopathy in comparison to normal controls. Similar observation were also reported in diabetics (Loven et al., 1986, Murakami et al., 1989). But Matkovics et al. (1982) reported that there was no significant difference between
normal and diabetics. Inter-group comparison shows that there is no significant difference between diabetic with or without retinopathy, BDR and PPDR. This shows that GR has no further role in the progress of retinopathy.

Decrease in the activity of GR may be mainly due to the polyol pathway. AR, the first enzyme in polyol pathway also requires NADPH as a cofactor. Therefore AR and GR compete for NADPH and the former utilises more NADPH than the latter resulting in decreased activity of GR. Gonzalez et al. (1986) pointed out that AR has a Km value for NADPH ten-fold lower than GR. So AR can utilise more NADPH from GR which results in the disturbance of GSH formation (Yeh and Ashton, 1990). Crucht et al. (1996 and 1997) reported that there is increased activity of polyol pathway in diabetics. Eye tissues of diabetic shows an increase in the activity of polyol pathway, especially in retina (Hotta et al., 1991). This increase in the activity of polyol pathway plays an important role in the impairment of glutathione redox status in diabetics (De-Mattia et al., 1994). De-Mattia et al. (1994) support the hypothesis that activation of polyol pathway results in decreases in NADPH and GSH. Thus inhibition of polyol pathway bring about decrease in oxidative stress in diabetics, thereby preventing the formation of retinopathy. The present study showed that in diabetic retinopathy, NADPH production was very low, i.e., decrease in the activity of G-6-PD (Table 4.7) and 6-PGD (Table 4.8). Similar observations were also reported in diabetics by Costagliola (1990) and Muggeo et al. (1993). Inter-group comparison revealed that G-6-PD activity is significantly decreased in diabetic with retinopathy in comparison to without retinopathy. But in between BDR and PPDR, no significant difference was obtained. In case of 6-PGD, diabetic retinopathy showed a significant decrease in comparison to normal controls. Muggeo et al. (1993) reported that there was no significant difference of 6-PGD in obese patients with or
without diabetes. But in diabetics with or without retinopathy 6-PGD fails to show any significant role in retinopathy.

The low activity of G-6-PD in retinopathic cases results in the reduced conversion of GSSG to GSH as observed in diabetic patients by Costagliola (1990). TK and TA activity showed a significant decrease in diabetic with or without retinopathy in comparison to normal controls. Inter-group comparison showed that TA activity was insignificant, but that of TK was significantly changed in diabetic with retinopathy in comparison to those without retinopathy. Reduction in TK and TA activity indicated that both of them had important role in the development of diabetic retinopathy, especially that of TK. The decrease in TK and TA activity might affect normal metabolic pathway of HMP shunt resulting in decreased synthesis of pentose phosphate, which is essential for nucleic acid synthesis and thereby protein synthesis.

TK is a thiamine dependent enzyme and requires thiamine pyrophosphate as a coenzyme. So deficiency of thiamine may cause inactivation of TK and inhibition of the recycling of glucose through hexose monophosphate shunt. In red cell, the combined activity of TK and TA provides an active system for the inter-conversion of pentoses and hexoses. Although a portion of ATP is formed via oxidation through the HMP, a major part of it is derived from glycolysis (Lionetti, 1974). So any defect in the HMP shunt can also contribute towards the disturbance in the level of ATP. Thus decreased TK and TA activity in the present study points to the fact that their role in the damage of retinal tissue and the development of diabetic retinopathy is of importance. Low levels of TK in diabetes with Alzheimer disease was reported by Lonsdale (1988). In addition to this, he also observed that the nutritional supplementation of thiamine hydrochloride, Mg and K aspartate, vitamin complexes etc. was able to regain the normal activity of TK along with the
removal of blurred vision. But Nixon et al. (1990) reported that the enzyme deficiency cannot be a consequence of nutritional deprivation.

GSH, an antioxidant plays an important role in the protection of membranes against oxidative damage, maintenance of membrane protein integrity and ascorbic acid in reduced form (Murakami et al., 1989). Table 4.12 clearly shows that there was a significant decrease in GSH in diabetic with or without retinopathy in comparison to normal controls. Similar observation was also observed in diabetics by Gandhi and Chowdhury (1979), Uzel et al. (1987) and Ciuchi et al. (1996) in erythrocytes of diabetic patients and diabetic rats. Inter-group comparison also revealed that GSH activity was significantly decreased in diabetic with retinopathy in comparison to without retinopathy. Reduction of GSH in redox state thus favours the oxidation of critical -SH on the membrane, resulting in the oxidative inactivation of -SH dependent proteins. Such a link between cellular GSH content and membrane -SH has also been demonstrated in G-6-PD deficient erythrocytes in which NADPH levels are very low (Kosower et al., 1982). The present work confirms to the above observation.

Low levels of GSH in retinopathy may be due to the decreased activity of G-6-PD (Table 4.7) which in turn results in decreased production of NADPH. The NADPH is later on used by GR for conversion of GSSG to GSH as observed by Kosower et al. (1982) and Paolisso et al. (1992). Due to the decrease in GSH, lipid peroxidation is increased which leads to the damage of tissue, especially retina resulting in retinopathy. There was also an increase in MDA and DC in the present study (Tables 4.5 and 4.6). This was in agreement with the observation of Kaji et al. (1985) and Bambolkar and Sainani (1995). Kakkar et al. (1995) reported that oxidative stress can lead to microvascular complications. In addition to this Crabbe et al. (1980) was of the opinion that oxidation in retinopathy was two-fold higher
than in diabetic cataract. This confirms the role of low levels of GSH in PPDR than BDR. Therefore, this significant decrease in GSH in PPDR may lead to severe damage to the retina in diabetic retinopathy. MDA and DC levels were also found to increase in retinopathic condition leading to decrease in GSH.

GSH takes part in oxidation-reduction reactions, hydrogen ion transfer, photoionization, electron transfer to metal ions and displacement and addition process (Meister and Anderson, 1983). Reduction of $\text{H}_2\text{O}_2$ to water is catalysed by GSH-Px and GST with the conversion of GSH to GSSG. GSH is also converted to GSSG by transhydrogenation. So the increased utilisation of GSH by GSH-Px and GST may cause decrease in GSH. Paolisso et al. (1992) reported that GSH plays a role in glucose induced insulin secretion. But in the present study, the decreased activity of GSH fails to induce insulin secretion which in turn fails to protect cellular structure.

GST is a multi-functional protein involved in the detoxification of an extensive array of compounds with the help of GSH (Meister and Anderson, 1983). In the present study, there was a significant increase in the activity of GST in diabetics with or without retinopathy in comparison to normal controls. Similar observations were also reported in diabetics by Sureshkumar and Menon (1992), and Raza et al. (1996). Gupta et al. (1993) and Suchocka et al. (1995) that the ability of GST was decreased in diabetic rats. But Yoshida et al. (1995) reported that there is no significant change in the activity of GST in diabetic patients as compared to normal controls. This showed that in diabetic condition, there should be an increase in the harmful electrophilic compounds and peroxidation resulting in increase in the activity of GST. It has been observed that GST possesses peroxidase activity and participates in the reduction of fatty acid hydroperoxides to non-toxic alcohols (Awasthy et al., 1980; Singhal et al., 1992). This function is
mainly carried out by GSH-Px. In the present study, difference in activity of GST was insignificant between diabetic with or without retinopathy, but in the case of GSH-Px, the difference in activity was highly significant. This shows that in the progress of diabetic retinopathy, GST fails to exert any role.

The activity of GSH-Px and catalase was significantly elevated whereas the opposite is the case of SOD in diabetics with or without retinopathy in comparison to normal control. Increased activity of GSH-Px was observed in diabetic patients (Matkovics et al., 1982; Sundaram et al., 1996) and diabetic rat tissues (Dohi et al., 1988). While decreased activity of GSH-Px was obtained in diabetics (Hagglof et al., 1983; Uzel et al., 1987) and diabetic rats (Gupta et al., 1993). But Olczyk et al. (1994) failed to observe any significance.

Significantly increased activity of catalase was reported in diabetics (Rema et al., 1995) and diabetic rats (Matkovics et al., 1982; Wohaieb and Godin, 1987). But Sundaram et al. (1996) (human diabetics) and Gupta et al. (1993) (diabetic rats) reported decrease in the activity of catalase. Inter-group comparison also reveals that catalase and GSH-Px activity was significantly increased in BDR and PPDR in comparison to diabetics without retinopathy. Both PPDR and BDR showed significant difference between the groups.

The increased activity of catalase and GSH-Px may be due to the compensatory mechanism for scavenging excess $H_2O_2$ formed in the diabetic retinopathic cases. The present findings (Tables 4.5 and 4.6) is in agreement with increased levels of lipid peroxidation reported earlier (Jennings et al., 1991; Bambolkar and Sainani, 1995). Cohen and Hochstein (1963) demonstrated that at lower concentration of $H_2O_2$, the GSH-Px was very active while at higher concentration of $H_2O_2$, catalase activity was more. GSH-Px, on the other hand, is able to protect the red cells from the effects of catalase deficiency (Rapoport and
Due to the above reason, GSH-Px activity increased along with catalase activity during the increased production of \( \text{H}_2\text{O}_2 \). Thus for the protection of retina from toxic materials, the activity of catalase and GSH-Px was increased. In addition to this GSH-Px and catalase also protect SOD from inhibitory effects of \( \text{H}_2\text{O}_2 \) which in turn protect the retina from \( \text{O}^2_- \) and further formation of \( \text{OH}^- \) as in Figure 4.5. Erythrocyte GSH-Px is a selenium-dependent enzyme. Gebre-Medhin et al. (1984) observed an increase in the concentration of plasma selenium in diabetic children. This clearly shows that in diabetics, increased selenium content is an adaptive process of scavenging excessive free radicals especially \( \text{H}_2\text{O}_2 \).

The activity of SOD was decreased significantly in diabetic with or without retinopathy in comparison to normal controls. This was in agreement with the reported observation of Nath et al. (1984), Collier et al. (1990) and Sundaram et al. (1996). But Olczyk et al. (1994) failed to obtain any significance in it. PPDR cases also showed a decreased SOD activity in comparison to those without retinopathy. Rema et al. (1995) also obtained similar results. Decreased activity of SOD in retina of galactosemic rat was observed by Kowluru et al. (1997). It is clear from these facts that the decreased levels of SOD fails to remove \( \text{O}_2^- \) which in turn cause damage to the retina leading to retinopathy. Wolff et al. (1991) reported the inactivation of SOD due to the high \( \text{H}_2\text{O}_2 \) concentration. \( \text{H}_2\text{O}_2 \) can also be produced from other metabolic routes (Hone et al., 1981). Therefore decreased activity of the SOD observed in DR can result in an increased accumulation of oxygen free radicals. Arai et al. (1987) observed that hyperglycaemic state can leads to decrease in the SOD activity. Loven et al. (1983) observed that abnormal levels of SOD can be returned to normal in diabetics by insulin. But Corina et al. (1996) failed to mitigate either the incidence or the severity of OIR by supplementation of SOD. It is clear from the present study that
there was no significant difference in the activity of SOD in BDR and PPDR. All these results show that antioxidant deficiency and excessive peroxidative damage appear very early on the diabetic conditions leading to retinopathy which can be prevented by proper metabolic control at the early stages.