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Lipid peroxidation in diabetic retinopathy

Oxidative stress has been implicated in pathogenesis of diabetic retinopathy. It has been hypothesized that hyperglycaemia, the main sign of diabetes, may activate the diacyloglycerol pathway with an increase in protein kinase C activity, in non-enzymatic glycosylation and secretion angiogenic factors like VEGF, IGF and bFGF. The consequence of chronic hyperglycaemia and "glucose toxicity" is oxidant-induced free radical damage of diabetic retina and vascular endothelium. Lipid peroxidation is a self-perpetuating chain reaction, it supplies free radicals and induces cell damage and death (5, 7, 9, 10, 11, 14). In these reactions various molecular species are formed, like malondialdehyde and 4-hydroxynonenal, which are known to activate expression of VEGF and cause rheological disturbances. Retinal rod outer segments and vascular endothelium as they contain a high level of long-chain polyunsaturated fatty acids, become the objects of peroxidation (5, 12, 13).

The aim of the present study was to estimate lipid peroxidation by detecting the concentration of malondialdehyde and 4-hydroxynonenal in diabetic patients with retinopathy, subjects without retinopathy and the reference group.

MATERIAL AND METHODS

Sixty-one patients were studied, including 30 patients with severe non-proliferative retinopathy in NIDDM and 31 diabetic patients without retinopathy. The reference group were 11 age-matched, systemic healthy, cataract subjects. Exclusion criteria were smoking and renal complications.

Heparinized blood samples were centrifuged at 500 g for 5 min to obtain plasma. Concentration of malondialdehyde and 4-hydroxynonenal was measured in plasma sample using a Lipid peroxidation Assay Kit (Calbiochem-Novabiochem Corp.). This method is based on the reaction of MDA and 4-HNE with N-methyl-2-phenylindole and methanesulfonic acid at 45°C, which produce chromophore with maximal absorbance at 586 nm.

200 µl of plasma was mixed with 650 µl of N-methyl-2-phenylindole and a 150 µl of methanesulfonic acid was added. The sample was incubated at 45°C for 40 minutes. After cooling down on ice, the absorbance was measured at 586 nm. Results were expressed in µM/ml of plasma. Data were statistically analysed with independent t-Student test.

RESULTS

The concentration of lipid peroxidation products in patients with retinopathy was statistically significantly elevated in comparison to diabetic patients without retinopathy ($p < 0.001$) and the reference group ($p < 0.001$). We do not notice any significant differences in levels of MDA and 4-HNE between

patients without diabetic retinopathy and the reference group (p = 0.08). The results are shown in Table1 and Figure 1.

Table 1. Malondialdehyde and 4-hydroxynonenal concentration in plasma

	n	Mn ± SD	Me	Min	Max
Patients with retinopathy	30	20.13 ± 7.15	19.38	9.09	36.27
Patients without retinopathy	31	11.53 ± 2.82	10.9	6.77	16.8
Reference group	11	9.72 ± 3.09	9.23	4.91	13.72

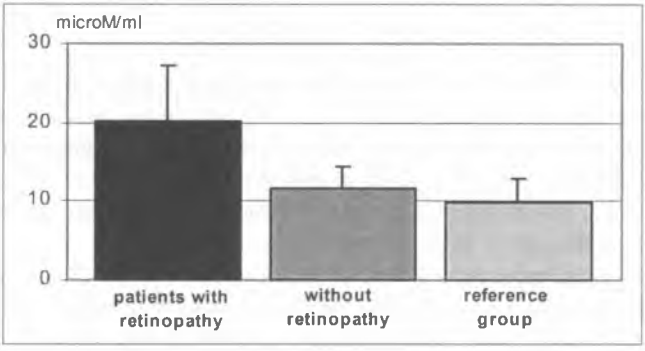

Fig. 1. Malondialdehyde and 4-hydroxynonenal concentration in plasma

DISCUSSION

More recently, lipid peroxidation has received a great deal of attention, and studies suggest its role in pathogenesis of diabetic retinopathy. The end products of lipid peroxidation are less reactive than free radicals and may diffuse in organism as secondary markers of oxidative damage of cells.

This study measured concentration of MDA and 4-HNE using a specific and modern method based on the reaction with N-methyl-2-phenylindole and methanesulfonic acid. In literature the products of lipid peroxidation (only MDA) were estimated with the method based on the reaction of MDA with thiobarbituric acid in low pH. As the result of this process they obtain red-coloured complex the concentration of which was measured fluorometrically. This classical reaction is sensitive but highly non-specific, because it is affected by many interfering agents. Gutteridge et al. suggest that the method with TBA may produce elevated results of MDA concentration by about 70 times in comparison with more specific methods (4).

In this study we obtained a significantly higher level of MDA and 4-HNE in the group of diabetic patients with retinopathy compared with patients without retinopathy and the reference group, and there were no differences between the group without retinopathy and the reference group. Comparable results were reported by other authors, although they used less specific method of measurement (1, 2). For example Losada et al. and Guzel et al. presented high concentration of MDA (and TBA-reactive species) in patients with IDDM (6, 8).

Higher concentration of MDA and 4-HNE in vitreous is postulated in proliferative diabetic retinopathy in comparison with non-affected vitreous. Verdejo et al. measured levels of lipid peroxidation products in basal state (Basal LPO) and after induction by NADPH-Fe (ILPO). In all cases they obtained statistically significant elevated concentration of MDA and 4-HNE in vitreous with proliferative retinopathy (15). Armstrong et al. reported that TBA reactive species increased 15 times after streptozocin induced diabetes in rats at day 22, and decreased at day 39 of experiment. They also noticed morphological damage in retina, rod outer segments and thickness of retinal pigment epithelium (3).

CONCLUSION

In view of our results we can conclude that oxidative stress measured as MDA and 4-HNE concentration using a specific and sensitive method is an important risk factor in the development of diabetic retinopathy.

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SUMMARY

Oxidative stress has been implicated in pathogenesis of diabetic retinopathy. It has been hypothesized that hyperglycaemia may damage vascular endothelium and retina by inducing the

synthesis of oxidant reactive species. The aim of the present study was to estimate lipid peroxidation by detecting the concentration of malondialdehyde and 4-hydroxynonenal in diabetic patients with retinopathy, subjects without retinopathy and the reference group. Sixty-one patients were studied, including 30 patients with severe non-proliferative retinopathy in NIDDM and 31 diabetic patients without retinopathy. The reference group were 11 systemic healthy patients. Concentration of malondialdehyde and 4-hydroxynonenal was measured in plasma sample using a Lipid peroxidation Assay Kit (Calbiochem-Novabiochem Corp.). The concentration of lipid peroxidation products in patients with retinopathy was statistically significantly elevated in comparison to diabetic patients without retinopathy ($p < 0.001$) and the reference group ($p < 0.001$). We do not notice any significant differences in levels of MDA and 4-HNE between patients without diabetic retinopathy and the reference group. In view of our results we can conclude that oxidative stress is an important risk factor in the development of diabetic retinopathy.

Peroksydacja lipidów w retinopatii cukrzycowej

Obecnie uważa się, że stres oksydacyjny bierze udział w patogenezie retinopatii cukrzycowej. Podwyższony poziom glukozy we krwi prowadzi do uszkodzenia komórek śródbłonka naczyń oraz siatkówki poprzez indukcję syntezy reaktywnych form tlenu. Celem pracy było oznaczenie stężenia malonylodialdehydu i 4-hydroksynonenalu w osoczu krwi chorych na retinopatię cukrzycową, chorych na cukrzycę typu 2 bez retinopatii oraz porównanie wyników z grupą referencyjną. Badaniem objęto 61 chorych, w tym 31 chorych z zaawansowaną retinopatią nieproliferacyjną oraz retinopatią proliferacyjną oraz 30 chorych na cukrzycę typu 2 bez retinopatii. Grupę referencyjną stanowiło 11 pacjentów bez chorób ogólnych. Stężenie malonylodialdehydu i 4-hydroksynonenalu oznaczano spektrofotometrycznie przy użyciu zestawu Lipid peroxidation Assay Kit (Calbiochem- Novabiochem Corp.). Stężenie produktów peroksydacji lipidów było istotnie statystycznie wyższe u osób z retinopatią w porównaniu z chorymi bez retinopatii ($p < 0.001$) i z grupą referencyjną ($p < 0.001$). Nie odnotowano istotnych statystycznie różnic w stężeniu MDA i 4-HNE pomiędzy grupą chorych bez retinopatii i grupą referencyjną. Wyniki badań pozwalają twierdzić, iż stres oksydacyjny, mierzony pośrednio jako stężenie MDA i 4-HNE, może brać udział w rozwoju retinopatii cukrzycowej.