

CONCENTRATION OF CONJUGATED DIENES IN PREGNANT WOMEN WITH INTRAHEPATIC CHOLESTASIS

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Intrahepatic cholestasis is a disease characterised by pruritus and increased liver function tests. The main aim of the study was to find correlation between cholestasis and lipid peroxidation products. The group studied consisted of 26 hospitalised pregnant women with intrahepatic cholestasis. The control group included 30 healthy pregnant women. A significant increase in the concentration of conjugated dienes was found in women with intrahepatic cholestasis (2.64 ± 0.62) with respect to the control group (2.46 ± 0.06 , mean \pm SD, $P < 0.05$).

INTRODUCTION

Intrahepatic cholestasis is a disease characterised by pruritus and stimulation of liver functions. One of the functions of the liver is inactivation of steroid hormones, produced by the mother and the fetus (Smolarczyk, Romejko, Wójcicka-Jagodzińska, Czajkowski, Teliga-Czajkowska & Piekarski, 1996; Thoma, 1996; Watkins, 1993) The initial reason of liver dysfunction is a decreased bile flow through hepatic lobule, which leads to bile concentration and cholestasis within intrahepatic bile ducts. This process is induced by progesterone, which causes also the atony of the gall bladder and bile ducts (El-Mir, Monte, Morales, Arevalo, Serrano & Marin, 1997).

Clinical practice shows that in many cases intrauterine fetal death occurs in pregnancies above 36 weeks of age, complicated by intrahepatic cholestasis. Recent studies showed that as a result of damage to hepatocyte, there is less glycogen available for the fetus than the fetal glucose demand. These observations have led to a hypothesis that a significant drop in the glucose level may be responsible for intrauterine acidosis of the fetus and intrauterine death.

Lipid peroxidation causes damage to cellular membranes, aldehyde peroxidation products are factors, which disorganise oxidative phosphorylation in mitochondria. As a result, the permeability of membrane to protons increases.

The formation of conjugated dienes is correlated with a shift of double bonds within polyunsaturated fatty acids, after detaching of a hydrogen atom in a free radical reaction. Their activity is thus correlated with the intensity of lipid peroxidation processes. Lipid peroxides can inhibit the synthesis of prostacyclin, and thus increase the aggregation of platelets and further intensify cellular oxidation processes. The main aim of the study was to find correlation between intrahepatic cholestasis and conjugated dienes as one of the main lipid peroxidation products.

MATERIALS AND METHODS

The study was done in the Clinic of Pathology in Pregnancy, Institute of Gynaecology and Obstetrics, Medical University of Łódź, in 1997–1999. The study group included all hospitalised pregnant women with intrahepatic cholestasis (26 cases). The control group consisted of 30 healthy pregnant women.

In all pregnant women lipid degradation products in the serum have been evaluated.

The examinations were done in the first day of hospitalisation. Blood samples of 10 ml were harvested from fasting subjects, from the cubital fossa. The blood was left at room temperature for 2 hours. After clotting the samples were centri-

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fused and the separated serum was placed in Eppendorf tubes.

To measure the level of conjugated dienes, 0.5 ml samples of serum were mixed with 7 ml of chloroform-methanol (1:2 vol/vol), shaken for 2 min and centrifuged ($2500 \times g$, 5 min). 5 ml of the lower (chloroform) layer was mixed with 2 ml of distilled water acidified with 0.1 M HCl to pH 2.5. The mixture was shaken again for 2 min and centrifuged ($2500 \times g$, 5 min). The chloroform layer was aspirated and dried under the flow of nitrogen. The residue was reconstituted with 1 ml of heptane and its absorbance was read against a heptane blank at 233 nm and measured in an Ultrospec 2000 spectrophotometer. The results are expressed in absorbance units per 0.1 ml of serum.)

RESULTS AND DISCUSSION

Due to methodological difficulties and very short half-life, direct determination of free radicals by spectrophotometry and chemiluminescence is done rarely. Instead, the activity of free radicals is evaluated on the basis of the concentration of the products of their activity (Wei, Calvin & Rogers, 1996). Figures 1 and 2 present the concentrations of conjugated dienes in the group of healthy pregnant women and of pregnant women with intrahepatic cholestasis, respectively.

In women with intrahepatic cholestasis the level of conjugated dienes was higher (2.64 ± 0.62 ; mean \pm SD) than in the group of women with normal pregnancy (2.46 ± 0.59 ; $P < 0.05$).

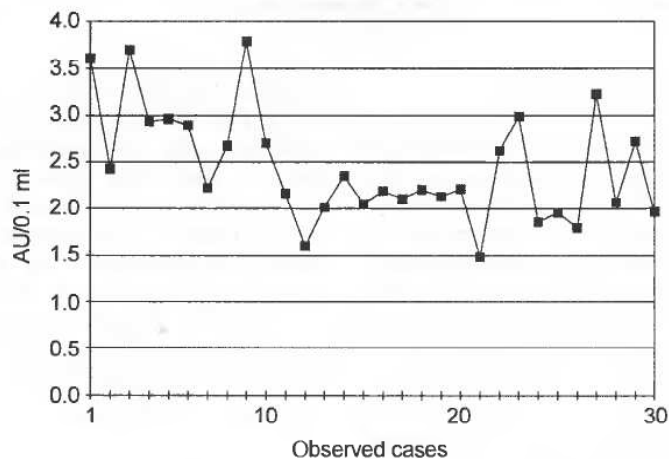


Fig. 1. Conjugated dienes in the control group

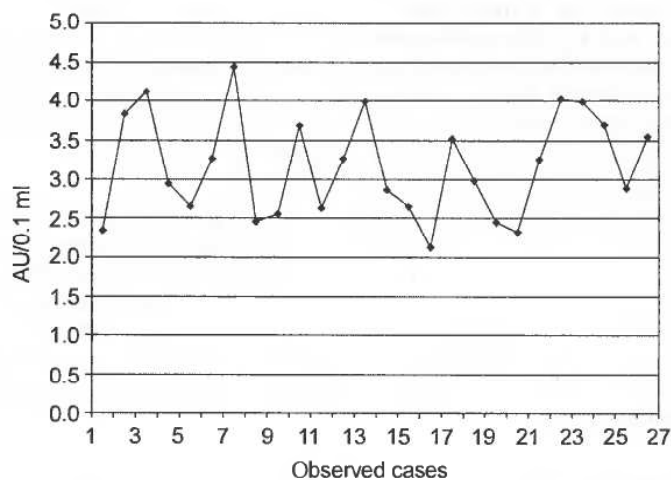


Fig. 2. Conjugated dienes in patients with cholestasis

The significant difference in the conjugated dienes concentration between the studied groups evidences an enhancement of the process of lipid peroxidation in cell membranes in cholestasis. Lipid peroxides, which have the possibility to inhibit the aggregation of platelets via the inhibition of prostacyclin synthesis, can affect the intracellular metabolic processes.

There is a close correlation between the increase of oxidative stress markers in the serum and the degree of cell membrane damage. Failure of compensatory mechanisms leads to irreversible damage to cellular membranes by changes in their permeability (Grieb, 1994; Lunec 1989). Free radical reactions give also products, which are not free radicals (reaction of termination). In the course of these reactions, residues of polyunsaturated fatty acids are decomposed (Bartosz, 1995).

According to Chen, Wilson, Gunning and Walker (1993) erythrocytes in preeclampsia have lower resistance to free radicals, which may point to an impaired antioxidative system. Therefore, cellular membranes may be damaged more easily. There is a direct correlation between the degree of damage to cellular membranes and increase in the level of these markers in the serum. Malondialdehyde, one of the aldehyde products of peroxidation, known to increase the permeability of mitochondrial membranes to protons, may be an important marker of risk of mitochondrial damage. According to Stipek and Machunowa, the serum concentration of MDA increased during delivery complicated by intrauterine hypoxia (Stipek & Machunowa, 1995). It means that cellular membrane oxidation is intensified.

The difference in the concentration of conjugated dienes between pregnant women with intrahepatic cholestasis and healthy ones was statistically significant. Increased concentration of conjugated dienes evidences activated and intensified reactions between fatty acids and alkyl radicals, which lead to the formation of lipid peroxides. Lipid peroxides can inhibit the synthesis of prostacyclin, and thus increase the aggregation of platelets and prompt further impairment of tissue oxygenation processes (Weiss, Turk & Needman, 1979). A significant increase of the level of these compounds in pregnancy complicated by hyper-

tension is connected with increased formation of free radicals in this obstetric pathology (Smith & Baker, 1994).

Acknowledgements

This work was supported by the Medical University of Łódź /502-11-564/105/4/KBN 4 PO5E 01616.

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