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# Determination of Free Fatty Acid Composition in Plasma Membranes of Neutrophils in Diabetics

**Abstract:** Since frequent and serious infections occur in diabetic patients, the investigation of the function of neutrophils is of importance. Any change in the lipid composition of the plasma membrane could be one of the causes of these infections. In order to investigate this possibility and to determine any abnormalities, neutrophils and monocytes were separated by the Histopaque-1119 method from the blood samples (20 ml) of 10 diabetic and 10 healthy persons. After disruption of the cells, membrane lipids and proteins were isolated. Free fatty acids of phospholipids in membrane lipids were isolated by hydrolysing with phospholipase B under an ultrasonic dismembranater. Free fatty acids were identified by gas chromatography at the chloroform phase. The results were compared with chromatogram of the standardised free fatty acids. It was established that there is a statistically significant difference in the plasma membrane free fatty acid composition of neutrophils between the patients and the control group (p<0.001). This study suggests a relationship between neutrophil dysfunction in diabetic patients and the lipid components of the neutrophil plasma membrane.

**Key Words:** plasma membrane, phospholipase B, free fatty acid, neutrophil

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## Introduction

Membranes are essential components of all cells. They form closed compartments arround cellular protoplasm to separate one cell from another, and thus permit cellular individuality. Membranes are complex structures composed of mostly lipids and proteins, and to a lesser extent carbohydrates. Membranes are constructed of a bilayer of amphipathic lipids made up of phospholipids and glycolipids. The relative amount of these two lipids varies with the type of membrane, even between the organellar membranes of the same cell (1).

The human body has a special protective system against toxic and infectious agents. This system consists of leukocytes, macrophages and lymphoid tissues. Neutrophils and monocytes are the leukocytes that destroy foreign agents by phagocytosis. While neutrophils function by reaching the area of infection via the bloodstream, monocytes function by passing tissues and forming the stationary and mobile macrophage systems, respectively (2,3).

It is known that patients with diabetes mellitus are more susceptible to infection than non-diabetic subjects of similar age, sex and socioeconomic background. Additionally, infections in diabetic patients are more severe and often more difficult to control. Despite these commonly held beliefs, the increased association of diabetes and infection has been very difficult to prove. The interrelationship between diabetes mellitus and infection is complex. Recent studies suggest that impaired leukocyte function may be responsible, in whole or in part, for the increased susceptibility of diabetics to certain bacterial and fungal infections. However, it is unknown whether humoral and cellular defects are responsible for this condition (4,5).

Under physiological conditions, membrane lipids are in a fluid state, which is essential for the occurrence of normal functions such as membrane biogenesis, trafficking, exocytosis and endocytosis. For the achievement of phagocytosis, the lipid composition of the membranes should be available in order to allow membrane fluidity. For example, the change in the length of fatty acids leads to a change in the thickness of lipid bilayer which, in turn, may affect the transition temperature at which the structure undergoes the transition from ordered to disordered. The longer and saturated fatty acids chains exhibit higher

transition temperatures, so membrane fluidity decreases. In other words, phospholipid composition and amount have a significant effect on the permeablity of a cell membrane (6).

The changes in the fatty acid content of phospholipids mentioned above may also occur in diabetes mellitus, which in turn may disturb the action necessary for phagocytosis. The changes in the lipid bilayer may also affect the functions of leukocytes. Thus, infections are frequently seen and increase in severity. However, in the literature review, we found no study concerning the lipid content of plasma membranes of leukocytes in diabetes mellitus.

The aim of this study was to investigate the possible relation between the membrane lipid content of diabetic neutrophils and functional disturbances. For this purpose, the type, amount, chain lengths, and saturated or unsaturated features of fatty acids of plasma membranes of neutrophils were investigated.

## Materials and Methods

After overnight fasting, venous blood samples were taken from 10 healthy and 10 diabetic subjects (age range: 17-68 years) and placed in heparinised tubes. Heparin was selected as an anticoagulant since EDTA or citrate, when used, could bind to divalent cations ($Ca^{+2}$, $Mg^{+2}$) on the surface of neutrophils and destruct the original structure of membrane (7). Neutrophils were separated by the Histopaque-1119 method. For the purpose of separation of lipids from proteins of neutrophil membranes obtained by the Histopaque-1119 method, firstly, the cells were destroyed by ultrasonication, the mixture was centrifuged and the supernatant was separated off. The precipitate containing membrane particles were treated with 1% Triton X-100 solution and thus lipids were separated from proteins (8).

Membrane total lipids were extracted by a modified technique (9). Phospholipids were purified by thin layer chromatography (TLC) on silica gel-G. The main advantage of TLC is to separate polar lipids for early separation of phospholipids from neutral lipids for early separation of phospholipids from other lipids. One mL of acetate buffer (0.5 M, pH:4) was added to that lipid component obtained. To this mixture, 10 mL of phospholipase B enzyme, which catalyses the hydrolysis of ester linkages in phospholipids, was added (10,11)

Hydrolysis was carried out by ultrasonication plus phospholipase B. In order to test whether hydrolysis of phospholipids is achievable or not, 1x5 cm commercially available TLC plates were utilised. Phospholipid (PL) spots seen before hydrolysis were not detected, thus indicating that both of the ester linkages had been hydrolysed. After that, the samples were mixed with 1 mL of chloroform. The chloroform phase obtained was put into special chromatography jars. Again, 1 mL of chloroform was added and the tube was shaken up and down 2-3 times. Then, chloroform was transferred into a glass jar and dried under nitrogen. Two hundred microlitres of chloroform was added and covered with a teflon-coated aluminium lid and stored at -70°C in deep freeze until assayed.

The samples were analysed by capillary gas chromatography (Tracor® 570 U.S.A.). The peaks obtained were evaluated by comparison with those of the comparison available standard specimen (Merck).

The data were given as mean ± standard deviation. For statistical evaluation, Student's t test was used. A p value lower than 0.05 was considered as significant (12).

## Results

The chromatogram of the PMN fatty acids of the control group is shown in Figure 1. In this chromatogram, there were significant peaks in undecanoic acid (11:0), heptanoic acid (7:0), oleic acid (18:1;9), stearic acid (18:0), pentadecanoic acid (15:0), linoleic acid (18:2;9,12), erucic acid (22:1;13), heneicasanoic acid (22:0) and petroselinic acid (18:1;12). In Figure 2, the chromatogram of PMN fatty acids of the patient group is shown. In this chromatogram, peaks in heptanoic acid (7:0), undecanoic acid (11:0), stearic acid (18:0), nonadecanoic acid (19:0), erucic acid (22:1;13), linoleic acid (18:2;9,12) and petroselinic acid (18:1;12) were detected. Although oleic, pentadecanoic and heneicosanoic acids were detected in the control group, these acids were not detected in the patient group. In contrast, while nonadecanoic acid was seen in the patient group, it was not detected in the control group. There were statistically significant differences between the membrane fatty acid compositions of the patient and control groups (p < 0.001) for all parameters.
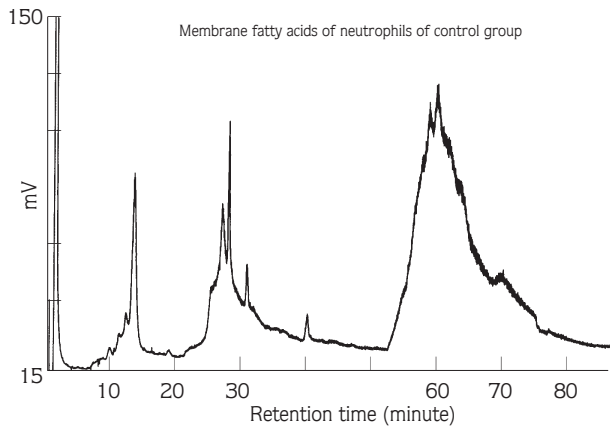
Figure 1a.   Chromatogram of membrane fatty acids of neutrophils of control group
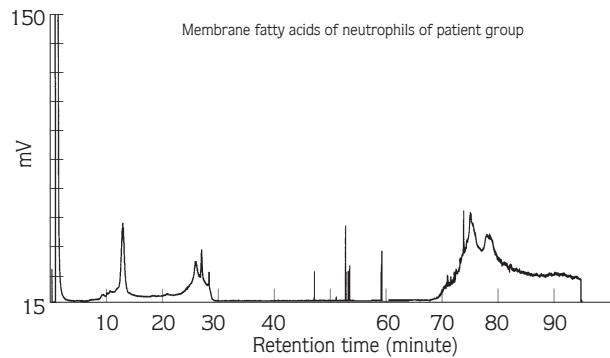


Figure 1b.   Chromatogram of membrane fatty acids of neutrophils of patient group
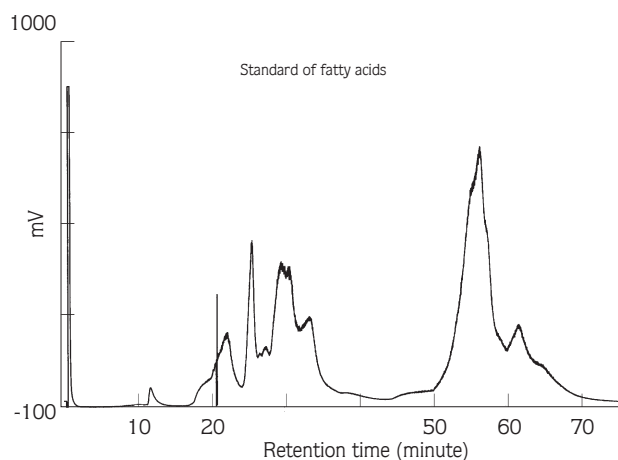


Figure 1c.   Chromatogram of fatty acid standards

## Discussion

From the findings of the present study, it was observed that the amounts of long-chain fatty acids, saturated fatty acids and even-numbered fatty acids were greater than those of short-chain fatty acids, unsaturated fatty acids and odd-numbered fatty acids, respectively. An evaluation was done to determine whether there was any difference between these two chromatograms (Table). In the comparison of the chromatograms of the patient and control groups, the patient group had higher amounts of heptanoic, linoleic, nonadecanoic acids and lower amounts of undecanoic, oleic, stearic, pentadecanoic, erucic, heneicosanoic and petroselinic acids than the control group had.

In earlier studies, it was reported that mobilisation of the PMNs of diabetic subjects were diminished (13). The chemotactic index was found to be decreased in the PMNs of diabetics, without any correlation being seen between the chemotactic index and the type of therapy or fasting blood glucose levels. In diabetic patients, particularly with poorly controlled diabetes, phagocytotic function is impaired and resistance to infection decreased. Membrane fatty acid composition determines membrane structure, membrane fluidity and mobilisation ability of a cell. Thus, it could be said that cell function might be affected, directly or indirectly, by membrane fatty acid composition (14).

These results suggest that altered lipid composition of leukocyte membranes may be a factor making diabetic patient prone to infection.

Table.   Percentage of fatty acids in neutrophil membranes of the control and patient groups.

| Fatty acids | Patient group | Control group | p values |
|---|---|---|---|
| Heptanoic (7: 0) | $5.42 \pm 0.67$ | $3.45 \pm 0.18$ | < 0.001 |
| Undecanoic (11: 0) | $2.47 \pm 0.25$ | $11.69 \pm 0.83$ | <0.001 |
| Pentadecanoic (15: 0) | $0.001 \pm 0.0003$ | $0.39 \pm 0.09$ | <0.001 |
| Oleic (18:1; 9) | $0.001 \pm 0.0003$ | $10.17 \pm 0.42$ | <0.001 |
| Linoleic (18:2 ; 9,12) | $25.91 \pm 0.60$ | $24.04 \pm 0.16$ | <0.001 |
| Stearic (18: 0) | $0.35 \pm 0.06$ | $4.55 \pm 0.12$ | <0.001 |
| Nonadecanoic (19: 0) | $0.24 \pm 0.05$ | $0.001 \pm 0.0003$ | <0.001 |
| Erucic (22:1; 13) | $0.24 \pm 0.11$ | $22.93 \pm 0.52$ | <0.001 |
| Heneicosanoic (22: 0) | $0.001 \pm 0.0003$ | $11.23 \pm 0.08$ | <0.001 |

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