Polynuclear Neutrophil Variation and Oxidative Stress in Type 2 Diabetics

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Abstract

Context: Chronic hyperglycemia and inflammation in type 2 diabetics may be correlated with oxidative stress, which is the basis of micro- and macro-angiopathic complications. The evolution of this inflammation resulted in the accumulation of neutrophils at the inflammatory site, the latter are in the basal state in a state of constant pre-activation for the production of O2. Objectives: This study aimed to determine the quantitative and morphological variation of neutrophils; and to identify the level of correlation between neutrophil levels and oxidative stress in type 2 diabetics in Lubumbashi. Material and methods: Spectrophotometric and microscopic methods were used to evaluate glycemia and neutrophils in the samples. Results: The study included 27 confirmed type 2 diabetics, including 52% of women. Their average age was 56.3 ± 11.79 years old. An increase in neutrophil count and a deviation of the Arneth curve were observed in 37.03% and 71.14%, respectively, of diabetic subjects. Conclusion: This study showed an increase in neutrophils in type 2 diabetics. This may be related to an indirect activation of the immune system in these subjects.

Keywords: Leukocytes, Oxidative status, Blood glucose, DRC.

INTRODUCTION

Diabetes is recognized as an important risk factor for a variety of intracellular bacterial infections, but research into deregulated immune mechanisms contributing to alteration of host-pathogen interactions is not well understood [1]. Diabetes is characterized by a chronic state of inflammation due to activation of pro-inflammatory mediators and increased formation of end products of advanced glycation. Increased oxidative stress also exacerbates chronic inflammatory processes seen in diabetes [1, 2].

Prolonged exposure to hyperglycemia is the primary factor causing vascular changes in type 2 diabetes. Chronic elevation of blood glucose levels results in the formation of advanced glycation products. These correspond to a heterogeneous class of glycosilated proteins and lipids found in plasma and vascular tissues [3, 4].

These advanced glycation products may cause severe inflammatory disorder with continued activation of neutrophils. This activation can lead to morphological alteration of neutrophils and the appearance of immature neutrophils in the general circulation [2, 5].

The neutrophils play an essential role in the inflammatory reaction and in the response to an infection. They are one of the first lines of defense against pathogens and during inflammation. Their immunological capacity is linked to their property of migrating to the inflammatory site and phagocytizing the pathogen or killing it via their microbicidal activity [4, 6].

However, several studies have shown a significant reduction in the phagocytic and microbicidal activity of neutrophils in type 2 diabetics. It has been shown that the reduction of blood glucose by insulin treatments in rats can significantly improve the phagocytic capacity of neutrophils [3].

During inflammatory oxidative stress in type 2 diabetics, tissue-resident macrophages and recruited neutrophils produce inflammatory mediators by activation of the Toll-like receptor 4 (TLR4) receptor /
nuclear kappa B receptor signaling pathway (NF-kappaB). These mediators include inflammatory cytokines and reactive oxygen species which, in turn, sensitize nociceptors and cause inflammatory pain \[1\].

Oxidative stress is defined as an imbalance in the balance between the production of reactive oxygen species (oxidants) and antioxidant defense systems, in favor of the former. It is the consequence of this imbalance which is at the base of the appearance of the often irreversible damages at the level of different systems of the organism \[7, 8\].

Reactive oxygen species are all chemical entities composed of free radicals derived from oxygen: superoxide anion (\(O_2^-\)), hydroxyl radical (\(^*\)OH), hydroperoxy radical (\(HO_2^-\)), peroxyl radical (\(RO_2^-\)), alkoxy radical (\(RO^-\)) and other non-radical species derived from oxygen: hydrogen peroxide (\(H_2O_2\)), hypochlorous acid (\(HOCl\)), ozone (\(O_3\)), singlet oxygen (\(1O_2\)), peroxynitrite (\(ONOO^-\)), which are not reactive but can be precursors of free radicals \(\[7\]\).

The aim of our work was to evaluate the variation in neutrophil count and morphology in type 2 diabetics, taking into account the oxidative stress underlying microangiopathic and macroangiopathic complications in these subjects.

**ENvironments, MATERIALs AND METHODS**

i) Study areas

Two executives served us for our experimentation. This is the center of diabetology CEDIA / Mellitus, this center was chosen because of the permanence of diabetic patients. The second medium was the CEFA-CEFR ASBL medical center; the latter was chosen because of the presence of an optical microscope coupled to the computer via a camera, which makes it possible to collect the images.

ii) Study material

The blood of type 2 diabetics was the main material of this study. It is on this blood that all biomedical analyzes have been performed.

iii) Methods of the study

Our study was prospective, based on the evaluation of the morphology and quantity of neutrophils in type 2 diabetics in Lubumbashi. Included in this study was any diabetic known and regularly monitored at the CEDIA / MELLITUS center; and having given free and informed consent to participate in the study. Spectrophotometric, hematological and histological techniques were used to evaluate blood glucose, neutrophil counts and neutrophil morphology, respectively \(\[9, 10\]\).

The Arneth curve was also used in the evaluation of neutrophil morphology, according to the following elements:

**Table 1**: Distribution of neutrophils according to the number of lobes of nuclei according to the Arneth curve \[\[11\]\].

<table>
<thead>
<tr>
<th>Number of lobes</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil levels</td>
<td>5 %</td>
<td>35 %</td>
<td>40 %</td>
<td>15 %</td>
<td>5 %</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

After biomedical analyzes, some important facts emerge in the results of patients with type 2 diabetes.

<table>
<thead>
<tr>
<th>Type of complication</th>
<th>Absolute number</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTA</td>
<td>3</td>
<td>11.10</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>10</td>
<td>37.03</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>48.13</td>
</tr>
</tbody>
</table>

Table 2 shows that of the 27 diabetics, 13 of them presented at the time of the analysis of complications. The results reveal that 11.10% of these diabetics had macroangiopathic complications and 37.03% of microangiopathic complications. Based on these results, we believe that these patients were in a state of oxidative stress \(\[2\]\). Several researchers have shown that in people with type 2 diabetes, the cause of complications is chronic hyperglycemia, which in turn leads to oxidative stress \(\[7, 12\]\).

**Table 3**: Distribution of neutrophils of diabetics according to the Arneth curve

<table>
<thead>
<tr>
<th>Direction of the curve</th>
<th>Absolute number</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>19</td>
<td>70.30</td>
</tr>
<tr>
<td>Normal</td>
<td>3</td>
<td>11.10</td>
</tr>
<tr>
<td>Right</td>
<td>5</td>
<td>18.50</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>100</td>
</tr>
</tbody>
</table>

Considering Arneth's curve which allows neutrophils to be distributed in relation to the number of lobes in their nucleus. It appears in Table 3 that in 70.30% of patients, the Arneth curve was deviated to the left; 11.10% of patients, the curve was normal and finally, in 18.50% of patients, curved was deviated to the right.

Laifer (2011) has established in his research a white formula during infections. He showed that the deviation of the Arneth curve to the right or left also depends on the degree of maturity of the neutrophils. A deviation of this curve towards the left side indicates the presence of the young neutrophils following a continuous activation of the neutrophils notably that which is met during the oxidative stress \[11\]. This corroborates our results, which showed 70.30% of the patients under a state of oxidative stress, since the curve was deviated to the left. So we think that among our diabetic patients, there were those who showed no signs of microangiopathic complications such as macroangiopathic, but who were still already in a state of oxidative stress \(\[9, 13\]\).

**Table 4**: Distribution of diabetics according to the neutrophils rate

<table>
<thead>
<tr>
<th>Neutrophil level</th>
<th>Absolute number</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increases</td>
<td>10</td>
<td>37.08</td>
</tr>
<tr>
<td>Normal</td>
<td>13</td>
<td>48.14</td>
</tr>
<tr>
<td>Inferior</td>
<td>4</td>
<td>14.8</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>100</td>
</tr>
</tbody>
</table>

The results shown in Table 4 show that in 37.08% of diabetics the rate of neutrophils was greatly increased, ie higher than the reference rate; while 14.8% of diabetics had a lower than normal rate.

This phenomenon can be explained by the metabolic disorders caused...
by chronic hyperglycemia, so often linked to inflammatory processes in type 2 diabetics. These disturbances are therefore responsible for the continuous activation and deactivation of neutrophils; and as a result, there will be fluctuations in neutrophil levels [5, 6, 12].

In addition, endogenous and exogenous pro-oxidants trigger activation of leukocytes and host defenses. These mechanisms interact in a cycle responsible for the control of oxidative / antioxidant homeostasis [8].

The functional activity of neutrophils was evaluated by the chemiluminescent method with a double successive stimulation by soluble stimuli with different mechanisms of action: phorbol-12-myristate-13-acetate (PMA) and phormyl-methionyl-leucyl-phenylalanine (fMLP) [8].

Deposition of advanced glycation products in diabetic rat skin has been shown to activate neutrophils prior to injury [13].

In addition, Tian et al (2016) showed that in vitro, exposure to advanced glycation products inhibited neutrophil viability, promoted cellular apoptosis, and prevented neutrophil migration.

Neutrophils in contact with advanced glycation products have shown increased secretion of inflammatory cytokines and increased oxidative stress.

The development of diabetes mellitus was accompanied by a violation of neutrophil and lymphocyte proliferation, an increase in myeloperoxidase activity and an improved process of apoptosis [5].

Our study found that 48.41% of diabetics had an impaired membrane neutrophil count of less than 10%. In addition to this, it has also been shown that no one has presented the level of the altered membrane neutrophils greater than 30%. In view of this result we find that in diabetic types 2, the membrane is not really affected (Figure 1). This result does not seem to support the hypothesis that oxidative stress could lead to apoptosis phenomena that can manifest itself by a membrane alteration of neutrophils [1, 2].

Thom et al (2017) has shown that an interdependent oxidative stress response to hyperglycemia disrupts the stability of the neutrophil cytoskeleton leading to myeloperoxidase production and IL-1β synthesis.

Myeloperoxidase production has been shown to be high in diabetic patients, but the underlying cellular mechanisms are poorly understood. Thus it has been hypothesized that raising glucose above the physiological level would stimulate leukocytes to produce myeloperoxidases and activate the nucleotide binding domain, pyrin-rich leucine-rich inflammasome 3 (NLRP3) [4].

Nagalievska et al, (2018) have shown that impaired leukocyte function is the factor causing susceptibility of patients with diabetes mellitus to infections. This being so, we still think that there is at least a possibility that the leucocytes will see their function altered, if not their membrane.

Note also that normalization of blood glucose levels using insulin prevents neutrophil recruitment and tumor growth. These results provide links between hyperglycemia-induced changes in neutrophil mobilization and primary tumor growth [3].

Figure 2: Neutrophils having a single-lobed nucleus

Figure 3: Neutrophils having a two-lobed nucleus

Figure 4: Neutrophils with a three-lobed nucleus

Figure 5: Neutrophils with a four-lobed nucleus

The images in Figures 2, 3, 4 and 5 give only the morphological and lobular idea of the neutrophils of our diabetic patients. However, diabetics have presented the number of different lobes; no explanation has been elucidated to date on the difference in the number of lobes.

CONCLUSION

Our study revealed that a good part of our diabetic patients presented neutrophil levels either increased or decreased; with a larger number of patients with an Arnet curve deviated to the left, thus reflecting a state of oxidative stress. The analyzes showed that the morphology of...
the neutrophils of the diabetics was not really affected. In the management of type 2 diabetes, particular attention should be focused on the normalization of blood glucose, which would prevent the deterioration of neutrophil function and morphology.

Limitation of the study

This study has known limitations in the size of the sample, which does not allow to extrapolate the results on all diabetics of Lubumbashi. From where to work on a large number of samples will allow drawing appropriate conclusions.

Contribution of the authors

All authors have contributed effectively to this research. They read and approved the final version.

Conflict of interest

The authors declare that there is no conflict of interest.

Thanks

Many thanks to the director of the CEDIA / Mellitus Diabetes Center for agreeing to provide us with samples from different diabetics; and the director of medical center CEFA-CEFOR ASBL to have accepted us in his laboratory for various biomedical analyzes.

REFERENCES