Biomedical Research and Therapy, 8(6):4417-4422

Open Access Full Text Article

Original Research

Neutrophils' functional activity in patients with mechanical jaundice and different levels of bilirubin

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ABSTRACT

Introduction: Mechanical jaundice (MJ) or bile duct blockage occurs when the bile ducts' patency is impaired, and the bile flow has stopped. One of the main pathogenetic factors developing complications with MJ is immune system imbalance, particularly its phagocytic link. The purpose of the study was to understand neutrophils' functional activity dependence with different blood bilirubin levels in men with mechanical jaundice. **Methods**: Forty-seven middle-aged men with mechanical jaundice were divided into three groups depending on the bilirubin levels in their blood: less than 60 μmol/L (n = 10), 60 – 200 μmol/L (n = 20), and more than 200 μmol/L (n = 17). The control group consisted of 50 practically healthy men of the same age. The neutrophils' functional state was assessed using the methods of spontaneous and induced luminol-dependent chemiluminescence of neutrophils. **Results**: In the group of patients with mechanical jaundice and a bilirubin level of less than 60 μmol/L, there was an increase in the values of T max spontaneous by 96%, I max spontaneous by 44.81%, S spontaneous by 224.6%, T max induced by 19.9%, I max induced by 13.5%, and S induced by 140.3%. In the group with bilirubin levels from 60 – 200 μmol/L, there was an increase in the values of T max spontaneous by 86.8%, I max spontaneous at 47.7%, S spontaneous at 204.6%, I max induced at 28.3%, S induced at 445%, and activation index at 70%. The group with bilirubin levels more than 200 μmol/L showed an increase in the level of T max spontaneous by 85.9%, I max spontaneous by 53.4%, S spontaneous by 927.3%, I max induced by 28.6%, S induced by 1045%, and activation index by 92.3% compared with the control values. The intergroup differences were found in S spontaneous levels, which were higher in the group with more than 200 μmol/L bilirubin levels compared with the 60 – 200 μmol/L group and less than the 60 μmol/L groups by 216.9% and 237.3% respectively. **Conclusion**: The revealed changes characterize the functional activity of neutrophils' increase with an increase in the bilirubin levels in patients with mechanical jaundice.

Key words: Bilirubin, chemiluminescence, mechanical jaundice, neutrophils

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History

- Received: Mar 13, 2021
- Accepted: Jun 01, 2021
- Published: Jun 30, 2021

DOI : 10.15419/bmrat.v8i6.679

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INTRODUCTION

Mechanical jaundice (MJ) or bile duct blockage (code K83.1, according to ICD 10) is a clinical and morphological manifestation complex that occurs when the bile ducts' patency is impaired, and the bile flow from the liver to the duodenum has stopped [1]. This pathology is detected in 12 — 45% of cases of hepatopancreatoduodenal diseases, mainly in men [2,3]. Complications involving MJ include biliary tract disturbances, dysfunctional liver disorders, and numerous systemic disorders [4,5]. One of the main pathogenetic factors that develop complications with MJ is immune system imbalance, particularly its phagocytic link [6,7]. This is manifested through phagocytosis process violations, changes in interleukin levels, neutrophil degranulation, and oxidative stress development [8–11]. Neutrophils' functional activity depends on the intensity of the respiratory burst process, and intracellular metabolism [12,13]. The chemiluminescence (ChL) method allows the analysis of the features of a respiratory burst in spontaneous and induced states in various diseases [14]. An extremely important indicator for patients with MJ is their bilirubin level, which plays a crucial role in the disease progression [15]. It has been previously shown that the increased levels of serum bilirubin may be the reason for the bactericidal activity of neutrophils' impairment in patients with hyperbilirubinemia [16,17]. Studying the neutrophils' functioning mechanisms and their relationship with bilirubin levels allow to identify intracellular targets, which makes it possible to modulate the cells' reactivity. Neutrophil dysfunction's detection can contribute to MJ's differential diagnosis and prognosis. In this regard, our work aimed to study neutrophils' functional activity dependence with the bilirubin levels in men with mechanical jaundice.

Cite this article : A. Darenskaya M, I. Kolesnikova L, V. Smirnova O, G. Gubanov B, V. Kasparov E, I. Kolesnikov S. **Neutrophils' functional activity in patients with mechanical jaundice and different levels of bilirubin**. *Biomed. Res. Ther.;* 8(6):4417-4422.

METHODS

Design of the study

The study used data on men with MJ (n = 47; mean age — 52.02 ± 5.18 years) who were treated at the Research Institute of Medical Problems of the North Krasnoyarsk Science Center. The control group consisted of practically healthy men (n = 50; average age — 48.7 ± 3.9 years) who underwent a routine medical examination. The criteria for inclusion for the clinical and control groups were as follows: male, age – 45–59 years, and informed consent to participate in the study.Thecriteriafor exclusion for the groups included the following: severe somatic diseases, including tuberculosis and HIV infection as well as drug addiction, and refusal to participate in the study. The diagnosis of mechanical jaundice (MJ) is based on (determined by) a combination of clinical and diagnostic signs (pronounced pain syndrome, bilirubin levels, alkaline phosphatase levels, liver enzymes levels, liver ultrasound, etc.) and the existence of cholelithiasis. The patients with MJ were divided into three subgroups depending on the bilirubin levels in blood: less than 60 μmol/L (n = 10), 60–200 μmol/L (n = 20), and more than 200 μmol/L (n = 17) [18].

Ethics approval for research

Before beginning the study, the Ethics Committee of the Research Institute of Medical Problems of the North Krasnoyarsk Science Center had approved it (protocol № 7 of November 16, 2012). Ethical principles were observed in accordance with the Helsinki Declaration of the World Medical Association (1964, ed. 2013).

Biochemical measurements

The neutrophils' functional state was assessed at the time of the patients' admission to the hospital and before therapy. Peripheral blood from the ulnar vein was used. The method of neutrophils' spontaneous and induced luminol-dependent chemiluminescence (ChL) assessing was used [19,20]. ChL was assessed by the biochemiluminescence analyzer BLM-3607 for 90 minutes. The following parameters were analyzed: the time when the curve reached the maximum level of ChL intensity (T max spontaneous and T max induced), the maximum intensity of the c ChL (I max spontaneous and I max induced), and the square under the curve of spontaneous ChL (S spontaneous) and induced ChL (S induced). Luminol was used as ChL amplifier, and opsonized zymosan served as an inducer of a respiratory burst. We also used the activation index indicator — the ratio of S induced to S spontaneous.

Statistical procedure

Statistical processing of the material was done using Statistica on Windows 8.0 application software package (Stat SoftInc., USA, 2008), and it included methods for determining the proximity of the sample to the normal distribution law (visual-graphical method, Kolmogorov–Smirnov agreement criteria, and Lilliefors and Shapiro–Wilk tests). The Mann–Whitney rank test was also used to analyze the statistical significance of the revealed differences among quantitative data. The critical significance level was assumed to be $p < 0.05$.

RESULTS

In the group of patients with MJ and bilirubin levels less than 60 μmol/L, there was a statistically significant increase in the values of T max spontaneous by 96% ($p = 0.02$), I max spontaneous by 44.81% ($p = 0.015$), S spontaneous by 224.6% ($p = 0.015$), T max induced by 19.9% ($p = 0.04$), I max induced by 13.5% ($p = 0.01$), and S induced by 140.3% ($p = 0.015$) compared with the control values (**Figure 1**).

The group of patients with MJ and bilirubin levels from 60 to 200 μmol/L were characterized by a statistically significant increase in T max spontaneous by 86.8% ($p < 0.001$), I max spontaneous by 47.7% ($p < 0.001$), S spontaneous by 204,6% ($p < 0.001$), I max induced by 28.3% ($p < 0.001$), S induced by 445% ($p < 0.001$), and activation index by 70% ($p < 0.001$) compared with the control values (**Figure 2**).

The group of patients with MJ and bilirubin levels more than 200 μmol/L differed as well as the previous group with an increase in the level of T max spontaneous by 85.9% ($p < 0.001$), I max spontaneous by 53.4 % ($p < 0.001$), S spontaneous by 927.3 % ($p < 0.001$), I max induced by 28.6% ($p < 0.001$), S induced by 1045 % ($p < 0.001$), and activation index by 92.3% ($p < 0.001$) compared with the control values (**Figure 3**).

The intergroup differences related to S spontaneous, which was higher in the group with bilirubin levels of more than 200 μmol/L compared with the groups with bilirubin levels less than 60 μmol/L by 216.9 % ($p = 0.002$) and the group with 60–200 μmol/L bilirubin levels by 237.3% ($p < 0.001$).

DISCUSSION

The concept of respiratory refers to the process of increasing the synthesis of reactive oxygen species (ROS) by phagocytic cells, which occurs either during phagocytosis or under regulatory influences [21,22]. Primary and secondary ROS have a moderate bactericidal and regulatory effect and are synthesized

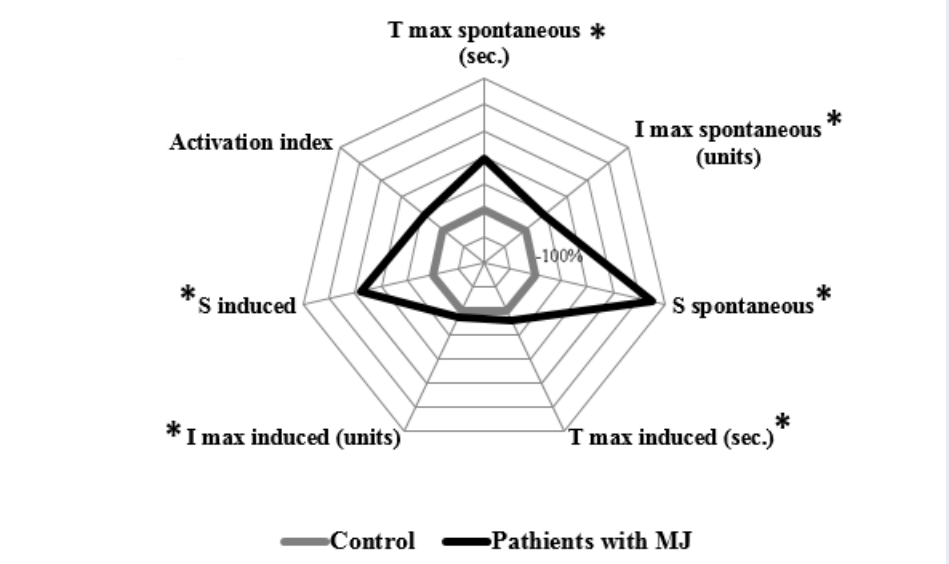

Figure 1: **Functional activity indicators of neutrophils in patients with MJ and bilirubin levels less than 60 μmol/L (*- statistically significant differences with the control (values taken as 100%))**.
Legend: **I**: intensity; **Max**: maximum; **MJ**: mechanical jaundice; **S**: square; **T**: time.

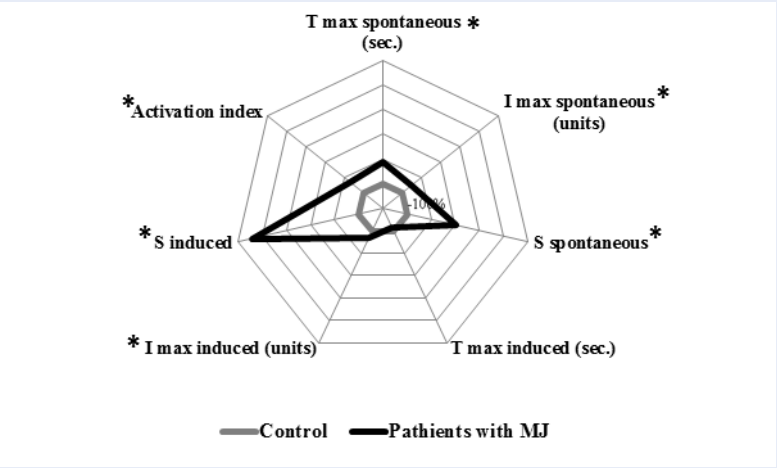

Figure 2: **Functional activity indicators of neutrophils in patients with MJ and bilirubin levels from 60-200 μmol/L (*- statistically significant differences with the control (values taken as 100%))**.
Legend: **I**: intensity; **Max**: maximum; **MJ**: mechanical jaundice; **S**: square; **T**: time.

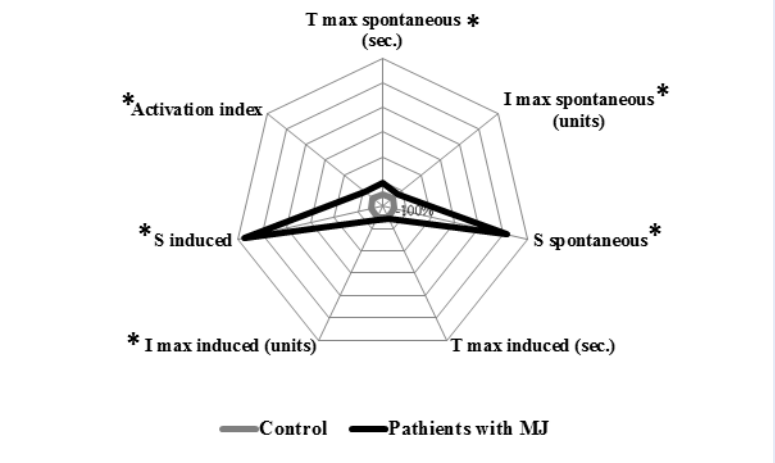

Figure 3: **Functional activity indicators of neutrophils in patients with MJ and bilirubin levels more than 200 μmol/L (*- statistically significant differences with the control (values taken as 100%))**.
Legend: **I**: intensity; **Max**: maximum; **MJ**: mechanical jaundice; **S**: square; **T**: time.

in cells during enzymatic or non-enzymatic reactions [22–26]. According to the literature, neutrophils' functional activity directly depends on the chemiluminescence's intensity; the higher the chemiluminescence, the greater their functional capacity [19]. Due to the low quantum yield of spontaneous luminescence, special luminescence acceptors, known as indicators, are actively introduced to sensitize luminescence. The ability of one such indicator, luminol, to interact with both primary and secondary ROS determines its inability to be used to assess particular ROS synthesis levels, but it allows us to integrally characterize the state of the respiratory burst of phagocytic cells [27]. In our study, an increase in T max was observed with spontaneous and induced chemiluminescence in patients with MJ and bilirubin levels less than 60 μmol/L. This indicator reflects the duration for the development of the maximum activity of ROS synthesis from the moment of antigenic induction of phagocytes' respiratory explosion and depends on the state of cells' metabolic reactions [12,28]. Their values, as a rule, decrease in the case of acute infectious and inflammatory diseases when neutrophils are in the activated state and increase in chronic inflammatory processes [29–31]. I max spontaneous and induced, reflecting the maximum synthesis of ROS by the cell, also increases at bilirubin levels below 60 μmol/L.

The square under the chemiluminescence curve, which integrally characterizes the entire complex of ROS (S) produced by phagocytes during the study period, changed in a similar way, with the most intense increase in spontaneous chemiluminescence. The group with bilirubin levels of 60–200 μmol/L was characterized by initial changes related to the control, while there was a tendency for a slight decrease in the values of a number of indicators.

The greatest increase was found in the S induced parameter, which characterizes the entire phagocytic pool of ROS, and was confirmed by the increase in activation index in this group. Activation index, which is the ratio of S induced to S spontaneous, shows the presence of intracellular metabolic reserves for the implementation of a respiratory burst [32]. The study revealed a significant increase regarding this parameter in patients with MJ and 60–200 μmol/L bilirubin levels. The group with MJ and more than 200 μmol/L bilirubin levels also showed an increase in indicators related to the control. However, in this group, there was also a significant increase in the values of S spontaneous compared with the group with lower bilirubin levels.

Bilirubin, belonging to bile pigments, is a product of the catabolism of heme-containing compounds and is mainly considered a negative factor in diseases associated with the liver and biliary tract [20]. The toxic effect of elevated bilirubin in blood on patients with MJ is manifested through the appearance of foci of necrosis in parenchymal organs, suppression of cellular immune response, and other effects [21,33]. The final stage of the pathological process in hepatic parenchyma is the initiation of hypoxic or free radical necrobiosis, subsequently causing cell death [1,2]. Despite the available data on the inhibitory ability of bilirubin in relation to neutrophil activity, we found an increase in

the total square under the chemiluminescence curve, which characterizes the entire complex of ROS (S spontaneous) produced by phagocytes. This fact can be associated with the benign genesis of the disease, when numerous pathological phenomena can be reversible, in contrast to the tumor process[3,8].

CONCLUSIONS

The changes in patients with mechanical jaundice reflect a significant increase in the neutrophils' functional activity in proportion to the bilirubin levels in the blood. These indicators can serve as additional laboratory markers that determine the course of the disease.

ABBREVIATIONS

ChL: chemiluminescence
I: intensity
Max: maximum
MJ: mechanical jaundice
ROS: reactive oxygen species
S: square
T: time

ACKNOWLEDGMENTS

None

AUTHOR'S CONTRIBUTIONS

Research concept and design: Darenskaya MA, Smirnova OV, Kasparov EV
Collection and processing of material: Gubanov BG, Smirnova OV
Text writing: Darenskaya MA, Smirnova OV, Gubanov BG
Editing: Kolesnikova LI, Kasparov EV, Kolesnikov SI
Approval of the final version of the article: Kolesnikova LI, Kolesnikov SI

FUNDING

The work was carried out within the framework of the state budgetary theme.

AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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