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Hematological Picture of Rabbits Immunized with *Pseudomonas aeruginosa*

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ABSTRACT

The current study was established to find out the role of immunization of *Pseudomonas aeruginosa*-whole sonicated antigen in adult white fur domestic rabbits. To achieve this goal, fifteen rabbits were allocated into 3 groups, the first group was immunized with *P. aeruginosa*–whole sonicated antigen and challenged with viable pathogenic *P. aeruginosa*; the second group (control negative) was treated with phosphate buffer saline and the third group was injected with viable pathogenic *P. aeruginosa* (control positive). The results demonstrated increasing levels of the measured parameters blood picture (total WBCs, lymphocytes, and granulocytes, RBCs and hemoglobin concentrations) in the first group compared with control negative group (T test was used). In contrast, a sharp fall was noted in total thrombocytes (platelets) count in the first group compared with control negative group. It can be concluded that immunization with *P. aeruginosa*– whole sonicated antigen may consider as a potent reproducible effective immunogen model for experimental immunological studies in rabbits.

Keywords: *rabbits, Pseudomonas aeruginosa, complete blood count*

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram negative rod-shaped encapsulated non spore forming bacteria and most strains are motile (1). *P. aeruginosa* emerged as an opportunistic pathogen, multidrug resistant microbe and most common in nosocomial infections (2). *P. aeruginosa* can infect animals and humans leading to a long list of infections which mainly include pneumonia, infected burn wounds, sinusitis, osteomyelitis, acute leukemia, cystic fibrosis, urinary tract, mastitis, organ transplants disorders and complications, sepsis, folliculitis, otitis, and ocular infections (3). Rabbits were used as good models to study *P. aeruginosa* infections as well as immunization in humans. For the above, rabbits considered as a successful model for pre-clinical studies to investigate immunization trials (4-7).

In fact, there are many difficulties to develop a successful vaccine against *P. aeruginosa* because of the complex structure of this microbe and presence of multiple virulence factors that enable this microorganism to cause a wide range of infections *in vivo*. Therefore, vaccines'

candidates and immunization trials targeted many compartments of *P. aeruginosa* such as external proteins, like flagella (8, 9) and pili (10), cytosolic protein (11), outer membrane proteins (12, 13), alginate which is an extracellular polysaccharide (14), lipopolysaccharide (15), capsular antigen (16), somatic antigen known as "O antigen" (17, 18), and biofilm (19).

The role of leucocytes against the infection with *P. aeruginosa* was studied by Al-Awadi and Alwan (20, 21) who found that polymorphonuclear cells and monocytes played the main important role in phagocytosing the bacteria. It was noticed that *P. aeruginosa* could cause harmful effect on RBCs causing apoptosis-like damage leading to shrinkage of these cells (22).

P. aeruginosa-whole sonicated antigen method was used in Iraq by Ati (23) to study the protective role of this antigen against arthritis in rabbits. This research is determined to study the effect of injecting *P. aeruginosa*-whole sonicated antigen in rabbits on complete blood picture as there are very rare or limited studies on sonicated *P. aeruginosa* and its impact on the blood picture.

MATERIALS AND METHODS

Animals and Experimental Design

All procedures conducted in this study was reviewed and approved by the scientific committee in the College of Veterinary Medicine, University of Baghdad in accordance with the ethical standards of animal welfare.

A total of fifteen adult white fur domestic rabbits both sexes about 1.8 Kg average body weight were fed on commercial pellets and grass for 14 days before the start of the experiment for acclimatization which housed in the animal house of the College of Veterinary Medicine, University of Baghdad, Iraq from April to July 2019. The strain of *P. aeruginosa* which was isolated from human infected burn skin was used in this research.

The animals were allocated into 3 groups, the first group was immunized with 1 mL of *P. aeruginosa*-whole sonicated killed antigen at a dose (1000 µg/mL) subcutaneously (S/C) at 2nd week of age (20), then the total protein concentration was estimated (24). A booster dose of 1 mL *P. aeruginosa*-whole sonicated killed antigen was injected S/C (1000 µg/mL) at 4 weeks of age, then challenged with 1 mL of viable pathogenic *P. aeruginosa* ($5x10^7$ CFU/mL) intraperitoneally (IP) according to (23) at week 6th then samples of 2.5 mL blood from the heart were collected after 2 weeks post challenge. The second group was injected with 1 mL of phosphate buffer saline (PBS pH = 7.2) at 2nd, 4th, and 6th week of age S/C and samples of 2.5 mL blood were collected from the heart at week 8th of age after the start of experiment (control negative). The third group was injected with PBS (pH = 7.2) at 2nd and 4th weeks (control positive). Challenge dose, 1 mL of $5x10^7$ CFU/mL *P. aeruginosa*, was injected intraperitoneally according to (23) at week 6th then samples of 2.5 mL blood from the heart were collected after 2 weeks post challenge to analyze the complete blood count (CBC).

Blood Samples

Blood samples were analyzed by blood analyzer apparatus (Mindray BC-2800, Auto Hematology Analyzer Shenzhen Mindray Bio-Medical Electronics Co., Ltd, China).

The following parameters were measured: total white blood cells (WBCs), lymphocytes, monocytes, granulocytes, hemoglobin, total red blood cells (RBCs), packed cell volume (PCV), mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width coefficient of variance, red cell distribution width standard deviation, platelets (thrombocytes), mean platelet volume, platelet distribution width, and procalcitonin test.

Statistical Analysis

GraphPad Prism package was used to apply the statistical analysis using T test for comparing between the groups of treatment and controls. The level of significance $P<0.05$ was considered in this study.

RESULTS

The results of the current research showed a significant ($P<0.05$) increase in the total leucocytes count including a sharp rise in lymphocytes count whereas granulocytes fell significantly ($P<0.05$) in their count. there was also remarkable expansion $P<0.05$ of total RBCs count, packed cell volume, Procalcitonin and lymphocytes count in the first group (immunized with 1 mL *P. aeruginosa*- sonicated whole killed antigen at a dose 1000 µg/ mL subcutaneously (S/C) at 2 weeks by comparison with control negative group. In contrast, there were significant drop $P<0.05$ in the count of mean platelets volume and platelets count in the first group by comparison with control negative group (Table 1).

Control negative group (injected with PBS) showed normal values in all blood parameters that have been measured. Whereas control positive group injected with1 mL PBS (pH = 7.2) at 2nd and 4th weeks, then challenge dose was injected by 1 mL *P. aeruginosa* ($5x10^7$ CFU/ mL) intraperitoneally illustrated remarkable rise $P<0.05$ in the count of lymphocytes and red cell distribution width coefficient of variance, but there was sharp decrease in granulocytes count, platelet distribution width, mean corpuscular hemoglobin, mean platelet volume, mean

corpuscular hemoglobin concentration, as well as procalcitonin.

DISCUSSION

Many decades ago, rabbits' blood components were clinically investigated by addressing the cell counts of neutrophils, lymphocytes, eosinophils, basophils as well as erythrocytes count and hemoglobin (25). In the current research, we extended blood parameters measured to include 19 parameters (Table 1). The main interesting finding from an immunological view is that an increased number of lymphocytes in the immunized group with *P. aeruginosa*. This result is in line with (26, 27) who found proteomic changes in the lymphocytes of rabbits infected with *P. aeruginosa* in sepsis.

There are very rare or limited studies on sonicated *P. aeruginosa* and its impact on the blood picture. In Iraq, Al-Awadi (28) studied the effect of immunization of *P. aeruginosa* in rabbits on the immune response and it was found that *P. aeruginosa* could cause a significant increase in the mononuclear cells (mainly monocytes and lymphocytes) in the blood which is nearly similar to what we identified except the monocytes which were found within the normal range of cell counts. Another study in Iraq was done by Al-Awadi (20) to study the protective role of *P. aeruginosa* outer membrane antigen against arthritis in rabbits and they found that mononuclear cells (mostly lymphocytes) and some neutrophils were severely infiltrated into the bone tissues.

Recently, scientists in the United States of America revealed normal percentages of white blood cells mainly lymphocytes 11.5% in the healthy young female New Zealand white rabbits (29), which is approximately similar to what we found in this study. However, rabbit hematology did not alter significantly when exposed to pathogenic methicillin resistant *Staphylococcus aureus* but stayed within the normal values (29) as we measured in this study.

Table 1. Immunized and challenged group with pathogenic *P. aeruginosa*

Parameter	Group1†	Group 2	Group 3
Leucocytes	12.3×10^9/Litre*	8.5×10^9/Litre	9.2×10^9/Litre
Lymphocytes	10.6×10^9/Litre*	2.7×10^9/Litre	7.5×10^9/Litre
Monocytes	0.6×10^9/Litre	0.3×10^9/Litre	0.5×10^9/Litre
Granulocytes	1.1×10^9/Litre*	2.3×10^9/Litre	1.2×10^9/Litre
Lymphocytes (%)	86.4%*	34.7%	81.5%
Monocytes (%)	4.5%	3.5%	5.4%
Granulocytes (%)	9.1%*	57.8%	13.1%
Haemoglobin	11 gram/decilitre	12 gram/decilitre	12 gram/decilitre
Erythrocytes	5.72×10^{12}/Litre*	5.77×10^{12}/Litre*	5.99×10^{12}/Litre
Haematocrit	38.8%	46.1%	44.5%
Mean Corpuscular Volume	68 femtoliter*	80 femtolitre	74.4 femtolitre*
Mean Corpuscular Haemoglobin	19.2 picogram*	20.7 picogram*	20 picogram*
Mean Corpuscular Haemoglobin Concentration	28.3 gram/decilitre	26 gram/decilitre*	26.9 gram/decilitre
Red Cell Distribution Width Coefficient of Variance	29.5%*	14%	20.7%*
Red Cell Distribution Width Standard Deviation	59.8 femtolitre*	53 femtolitres	56.9 femtolitre
Thrombocytes	35×10^9/Litre*	424×10^9/Litre*	36×10^9/Litre*
Mean Platelet Volume	6.6 femtolitre	6.6 femtolitre	7.1 femtolitre
Platelet Distribution Width	14.2	15.3	15.3
Procalcitonin Test	0.023%	0.0279%	0.025%*

* Means significant differences at a level ($P<0.05$). †Group 1: immunized and challenged with *P. aeruginosa*, Group 2: control negative (received phosphate buffer saline), Group 3: control positive (Non-immunized, challenged with pathogenic *P. aeruginosa*)

The results of the present work are concomitant with the findings of (30) who studied the blood picture in local breed rabbits immunized with whole sonicated *Corynebacterium bovis* antigen and found remarkable increase in the total WBCs number, lymphocytes by comparison with controls. Additionally, they found an increase in RBCs numbers and hemoglobin concentrations whereas, platelets declined.

The reasonable interpretation of leucocytes expansion after exposure to *P. aeruginosa*-whole sonicated killed antigen might be attributed to immune recognition to this bacterium through secretion of interferon-γ from the host cells (31-33). Both innate and adaptive immunity develop against *P. aeruginosa* antigens such as biofilm (34, Lipopolysaccharide (LPS) (35) or exotoxin A (36). This leads to an increase in the number of WBCs as an immune

response to *P. aeruginosa* (37) which is approximately similar to what it was found in this study.

Although *P. aeruginosa* is a Gram-negative bacterium which causes alterations to blood cells' count, the results of this study disagree with other results applied on rabbits by injecting other Gram-negative bacteria such as *Proteus vulgaris* which was found to cause a significant increase ($P<0.05$) in monocytes' count (38) whereas *P. aeruginosa* did not stimulate monocytes as shown in table 1. In the same line, *Proteus vulgaris* also used for rabbits' immunization showed elevated levels of all WBCs (total and differential counts except lymphocytes, which showed fluctuated results) after 4 weeks of exposure to whole sonicated antigens (39).

Comparing *P. aeruginosa* as a Gram-negative bacterium with other Gram-negative bacteria such as *Klebsiella pneumonia* which demonstrated severe increase in neutrophils and monocytes in the internal organs such as lung, liver, spleen, and intestine as described in the histopathological slides (40).

The conclusion of this research could be summarized by proofing an immunization effect of *P. aeruginosa*-whole sonicated killed antigen in rabbits

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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الصورة الدمية للارانب الممنعة بجراثيم الزوائف الزنجارية *Pseudomonas aeruginosa*

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الخلاصة

تم تصميم الدراسة الحالية لبحث دور التمنيع بجراثيم الزوائف الزنجارية *Pseudomonas aeruginosa* المستضد الكلي المكسر بطريقة الامواج فوق الصوتية في الارانب البالغة بيضاء اللون العترة المحلية. لتحقيق هذا الهدف تم استخدام 15 ارنب قسمت الى 3 مجاميع، المجموعة الاولى تم تمنيعها بالمستضد الكلي المكسر بالامواج فوق الصوتية لجراثيم الزوائف الزنجارية، المجموعة الثانية مجموعة سيطرة سالبة اعطيت محلول ملحي فوسفاتي مخفف، اما المجموعة الثالثة فقد اعطيت جراثيم الزوائف الزنجارية الضارية واعتبرت مجموعة سيطرة موجبة. اوضحت النتائج ارتفاع معايير الدم وتحديدا العد الكلي لكريات الدم البيضاء ، الخلايا اللمفاوية، والخلايا الحبيبية، بالاضافة الى ارتفاع كريات الدم الحمراء وتركيز خضاب الدم في الارانب الممنعة بالمجموعة الاولى بالمقارنة مع مجموعة السيطرة السالبة وقد استخدم اختبار T لغرض المقارنة الاحصائية. من ناحية اخرى، لوحظ انخفاض حاد في العد الكلي للصفيحات الدموية في المجموعة الاولى بالمقارنة مع مجموعة السيطرة السالبة. نستنتج من هذه الدراسة ان التمنيع بالمستضد الكلي المكسر بالامواج فوق الصوتية لجراثيم الزوائف الزنجارية ممكن ان يعطي استجابة مناعية قوية ومنتجة ونوصي باستخدامه في التجارب السريرية التجريبية المناعية في الارانب.

الكلمات المفتاحية: الارانب، الزوائف الزنجارية، العد الكلي لخلايا الدم