THE THROMBOCYTES AS AN IMMUNE INDICATOR

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ABSTRACT

This study investigated the effect of vaccine as an immune enhancing on chicken thrombocyte number. Also the relation between thrombocytes and other blood cells like erythrocytes and leukocytes were studied in regard to age. A total of 100 broiler chicks of one day old were used in this study. They were divided equally into two groups. One group was kept without vaccination and the other one was vaccinated twice against Newcastle disease at 7 and 20 days of age. During the whole experiment which is lasted for eight weeks, blood samples were collected daily for the first two weeks and weekly for the other six weeks. Thrombocytes, erythrocytes and leukocytes number were calculated for both groups. The results show a significant increase in thrombocyte number as well as in leukocytic number post vaccination. Beside that a high correlation coefficient is reported between thrombocyte and leukocyte in both vaccinated and control groups (99.8% and 97.5 %, respectively). The three cell types increase gradually from the first day of age and as the animal grows up. They seem to stabilize at the fourth week of age. On conclusion, these results investigate the role of thrombocytes in immunity. Certainly, the over all results reflect that we could depend on thrombocytes as an indicator of stress as much as the H/L ratio. It could be an indicator of the physiological and immunological state of the animal. Further studies are needed to investigate the role of thrombocytes in some immune-suppressive diseases.

Key words: Thrombocytes, Immunity.
الأقراس الدمية مؤشراً للاستجابة المناعية

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الخلاصة

نظراً لقلة البحوث التي تناولت العوامل المؤثرة على الأقراس الدمية من جهة ودورها الحقيقي في المناعة من جهة أخرى، صمم هذا البحث لدراسة تأثير اللقاح في الأقراس الدمية وكذلك الاستجابة المناعية للأقراس الدمية المتولدة بفعل اللقاح. كما تم استخدام معامل الارتباط بين الأقراس الدمية وخلايا الدم الحمر وخلايا الدم البيض وكذلك تأثير العمر على أعداد هذه الخلايا. تم استخدام 100 فحص لمجر 100 فحص يوم واحد. وربما الأقراس في غرفة مساحتها 60 م²، تشبه أرضية وأُسماً إلى مجموعتين متساويتين لقيمتها المجموع الأولى بلقاح لاسوساً لمرض النيوكاسيل مرتين بعمر 7 و 20 يوماً وترك المجموعة الثانية بدون لقاح. أُستمرت التجربة لمدة 8 أسابيع. تم سحب الدم من الوريد الجناحي يومياً للأسبوع الأول والثاني واسبوعياً للأسبوعين الستة البقية. حسب الأقراس الدمية وخلايا الدم الحمر وخلايا الدم البيض وأجري الفحص الإحصائي اللامز، أظهرت النتائج ارتفاعاً ملحوظاً لأعداد الأقراس الدمية بعد أخذ اللقاح مرتبطاً مع أعداد خلايا الدم البيض، كما أظهرت النتائج وجود عامل ارتباط عالي ووجود بين الأقراس الدمية وخلايا الدم البيض وخلايا الدم الحمر. أما عن تأثير العمر فقد أظهرت الدراسة أن الخلايا المدروسة تزداد تدريجياً بتقدم العمر وربما أنها ستتغير عند أربعة أسابيع. نستنتج من هذه الدراسة أن اللقاح سبب زيادة معنوية في عدد الأقراس الدمية وكذلك خلايا الدم البيض، أما تبين أن عدد الأقراس الدمية يأتي بالمرتبة الثانية بعد خلايا الدم الحمر وتزداد تقدم العمر متفاوتة مع الخلايا المدروسة. كما نستنتج التوصية على إعادة الاعتماد على الأقراس الدمية كدليل فلزلي ومناعي للمقاومة ضد الأمراض إذ أن دورها لا يقل أهمية عن دور نسبة H/L، وذلك تم أخذه كمؤشر لمقاومة الإجهاد. نوصي بإجراء دراسات مستقبلية عن دور الأقراس الدمية في بعض الأمراض التي تسبب نقص المناعة.
INTRODUCTION

Platelets or thrombocytes are minute fragment of cells consisting of a small amount of cytoplasm surrounding by a cell membrane. They help in hemostasis in all vertebrates(1). Avian blood lacks platelets, and their role in plug formation is assumed by thrombocytes(2). For a long time avian thrombocyte function was unknown and little work was carried out in this field. Nucleated chicken thrombocyte was described as small round to slightly oval cells with very condensed chromatin and they may resemble small lymphocytes(3). Later on avian thrombocytes had been reported as multifunctional cells that are involved in the uptake, sequestration and release of serotonin, hemostasis and phagocytosis(4). Serotonin which is found in a high amount in chicken thrombocytes is a precursor of melatonin hormone(5). This hormone had been reported to increase total leukocytic number and play an important role in maintaining the internal immunological environment(6). In addition to that avian thrombocytes play a role similar to that of mammalian platelets with regard to aggregation, clot formation. Thrombocyte has the ability to adhere to damaged endothelium and collect to form a hemostatic plug(4). Moreover it has been found that thrombocytes are the primary blood phagocytes in chicken(7). There was approximately three times as many thrombocytes as all other blood phagocytes combined (monocytes, heterophils and macrophages). Because thrombocytes have so many important functions in blood Gross(8) suggested that thrombocytes could also be used as a physiological indicator of the stress response much as the heterophil to lymphocyte ratio (H/L). Gross and Siegel(9) first noted that the H/L ratio is associated with stress and could considered more reliable indicator than plasma corticoids level. Al-Murrani et al.(10) first used the H/L ratio as a criterion for selection for stress resistance. This ratio was recommended to measure as an indicator for stress and criterion for selection for stress resistance when stabilized at about the age of three weeks(11). Because little is known about the effects of immunity on avian thrombocyte function, this study was designed for the first time to investigate the effect of Newcastle vaccine on thrombocytes number and to study their role in immunity as a response to vaccination. The relationship between thrombocytes and other blood cells which are erythrocytes and leukocytes was also investigated in regard to age progression.

MATERIALS AND METHODS

A total of 100 broiler Fawbro chicks were used in this study. Chicks were kept from day of hatching till the end of experimental period (56 days) in a 5 x 6 m room at the Animal House of the College of Veterinary Medicine, Baghdad University. They were fed normal starter and finisher broiler rations. Water and food were available ad libitum. Light was offered for 24hrs daily. They were divided equally into two groups. One group was vaccinated twice against Newcastle disease. Lasota vaccine was given orally to broiler chickens at 7 and 20 days of age. The other group remained without vaccination. The experiment lasted for eight weeks.

Blood samples were collected from the wing vein in heparinized tubes daily for the first two weeks and then weekly till the end of the experiment (8 weeks). Blood smears
were prepared and stained by Wright-Giemza stain for thrombocyte count (3). Then the number of thrombocytes were calculated according to this formula (12):

\[
\text{No. of thrombocytes} = \frac{\text{No. of thrombocytes counted in 5 fields} \times 35 \times 10^6}{1000}\]

Total red blood cells (RBC) and white blood cells (WBC) were counted by using a hemocytometer and Natt and Herrick solution (12). The RBCs pipette and the RBCs squares were used for both RBCs and WBCs count. In case of RBCs count, the blood was drawn to the mark 0.5 and the diluting fluid to the mark 101, therefore the diluting factor equal to 200. In case of WBCs count, the blood is drawn to the mark 1 and the diluting fluid to the mark 101, thus the diluting factor equal to 100. The following formula is applied:

\[
\text{No. of RBCs, WBCs} = \frac{\text{No. of cells} \times \text{diluting factor} \times \text{depth factor}}{\text{Area of calculated squares}}\]

The mean of the daily values during the first two weeks and the weekly ones for the other six weeks were statistically analyzed using the student's t-test to indicate the significance between vaccinated and control groups. The correlation coefficient between the studied parameters (thrombocytes, erythrocytes, leukocytes) of both groups were also analyzed (13).

**RESULTS**

Table (1) shows a significant difference in thrombocyte number between vaccinated and control groups (p<0.01). The leukocyte number also reveal a significant increase in the vaccinated groups (p<0.01). At the mean time, the vaccinated group shows no significant difference in erythrocyte number compared with control (p >0.01).

Table (2) shows a high positive correlation coefficient between thrombocyte and leukocyte number in vaccinated and control groups at 99.8% and 97.5%, respectively. We can also observe the correlation coefficient between thrombocyte and erythrocyte number in both vaccinated and control groups (63.4% and 86.7%) respectively. The leukocyte and erythrocyte number also shows a significant correlation coefficient in vaccinated and control groups (63.6% and 87.0% respectively).

Thrombocyte number (cell \( \times 10^9/L \)) shows a gradual increase from the first day till the fourth week. There was a significant difference (p<0.01) in the mean of thrombocyte number between the 1\(^{st}\), 2\(^{nd}\) and 3\(^{rd}\) weeks. Then it is stabilize after the third week. Vaccinated groups revealed a significant increase in the number compared with non vaccinated one Figure (1). The figure also shows a gradual increase in the total leukocytic number (cell \( \times 10^6/L \)) with age. There was a significant differences (p<0.01) in the total leukocytic number between the first three weeks of age as well as between the two groups. However, the Erythrocyte number (cell \( \times 10^{12}/L \)) increase from the first day. This increase continue as the bird becomes older. It seems to stabilize at the sixth week of age Figure (2). No significant differences between vaccinated and non vaccinated groups were reported.
Table (1): Represents the descriptive statistics between the experimental groups.

<table>
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<tr>
<th>N Statistic</th>
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<th>Mean Statistic</th>
<th>SE Statistic</th>
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<td>LC</td>
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Valid N 20

TV: Thrombocyte number for vaccinated group. TC: Thrombocyte number for control group. EV: Erythrocyte number for vaccinated group. EC: Erythrocyte number for control group. LV: Leukocyte number for vaccinated group. LC: Leukocyte number for control group.

Fig. (1): Represents the mean values of Thrombocytes and Leukocytes daily for the first two weeks and weekly for the other six weeks.

TV: Thrombocyte number for vaccinated group. TC: Thrombocyte number for control group. LV: Leukocyte number for vaccinated group. LC: Leukocyte number for control group.
### Table(2): The correlation coefficient between the experimental groups.

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** Correlation is significant at the 0.01 level ( 2-tailed ).

TV: Thrombocyte number for vaccinated group. TC: Thrombocyte number for control group.
EV: Erythrocyte number for vaccinated group. EC: Erythrocyte number for control group.
LV: Leukocyte number for vaccinated group. LC: Leukocyte number for control group.

**Fig.(2): Represents the mean values of Erythrocyte daily for the first two weeks and weekly for the other six weeks. EV: Erythrocyte number for vaccinated group. EC: Erythrocyte number for control group.**
DISCUSSION

Vaccinated group in this experiment shows an elevation in thrombocyte number. After vaccination to animals, there will be an activation of immune system to form antibodies in blood. The increasing number of thrombocytes is suggested to be due to the stimulating effect on IL₆. Thrombocyte maturation and differentiation is initiated by IL₆ (14).

Thrombocytes can readily phagocytose colloidal carbon. AC₃b-like receptor and analogs to mammalian platelet glycoproteins IIb and IIIa have been identified in chicken thrombocytes (15).

Thrombocytes showed morphological changes in response to stress and that these changes persist long after the stressor is removed (8). It is not surprising that thrombocyte play a similar role to H/L ratio and could have an important role in immunity. The results of this study reveal a significant increase in thrombocytes as well as leukocytes number as a defence mechanism in response to vaccination. Fenugreek seeds produces a significant increase in platelets number of rabbit beside globulin increase (16). This is suggested to be due to the direct effect of seeds on IL₆ which is responsible for platelets proliferation (14).

This study shows a gradual increase in thrombocytes number accompanied by an increase in total leukocytic and erythrocytic number from the first day of age till the eighth week. Avian thrombocytes are characterized by the presence of high amount of serotonin (5). Serotonin is a precursor of melatonin hormone which have been investigated to improve the immunological state when given orally to chicken through increasing lymphocyte number and decreasing H/L ratio, with increasing plasma globulin concentration as the birds grow up (6).

However, the elevation in total leukocytic number in the vaccinated group as well as with age is a normal result. This is done by an elevation of lymphocyte number with a decline in heterophil number. The decline in H/L ratio after melatonin administration is an indicator of stimulating the immune system of animal's bodies (6).

Al-Murrani et al (10) used the H/L ratio, in poultry, as a selection criterion for heat stress resistance. Heterophils increase on stress while lymphocytes decrease and thus they reported high H/L ratio in heat stressed chickens. It had been also reported that the chicks characterized by high body weight and those during early ages shows high H/L ratio because they suffer from physiological and metabolical stress. On the other hand, H/L ratio shows a gradual decline from hatch and seems to stabilize at about three weeks of age (11).

The increase in erythrocytes number with age is mainly due to increase body weight and O₂ demand by tissues. Sturkie (5) reported a high positive correlation coefficient between body weight and erythrocytes number. The increasing number of erythrocytes coincides with the decrease in reticulocyte number.

On conclusion, these results with our previous studies reported a special role of thrombocytes in immunity beside it's other roles. We conclude that thrombocytes certainly have a role in response to immunity and vaccine act as immune enhancer. Further researches on this subject must carried out to investigate the mechanism(s) of thrombocytes in maintaining the immunological state of the animal.
Acknowledgment

I wish to express my thanks to Professor Dr. W. K. Al–Murrani for his advice and help in the statistical analysis

REFERENCES


