Study of Some Immunity Characters Results of Injection antigenic *Staphyloccocus sp.* in Local Rabbits

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Abstract

This study was conducted to identify some immune manifestations resulting from the injection of staphylococcal bacterium antigens Heat-killing with a concentration of 0.75 ml in muscle and concentration 0.5 ml subcutaneous in local rabbits to see how immune response of these antigens achieve. Tests were carried out (late hypersensitivity, phagocytosis of candida albicans, and NBT dye reduction).

The results showed that the highest level of skin thickness was 9.50 ml compared to control 1.48 ml after 24 hours of antigen injection. The results also showed that the highest level of candida yeast is 81.30% when injecting concentration 0.5 ml under the skin compared to control 79.33%. As for the reduction of NBT dye, the highest level of dye reduction was 14.70% when the concentration was injected 0.75 ml with the muscle compared to 10.63% control.

We conclude that the injection of staphylococcus antigens (killed by heat) has the potential to induce immune response in local rabbits

***Keywords**: Staphyloccocus, Immunity, Rabbits*

Introduction

Staphylococcus is considered a cluster bacterium for its non-moving cluster shape and discolored according to gram, an anaerobic bacterium optional with the exception of sheep's Staph. Aureus. which is obligatory anaerobic [(1)] . It currently has approximately 20 or more types of skin injuries such as daisies and lobes and has a high pathogenic characteristic and contains an important pathogenic factor, coagulase. Bacteria are considered one of the most famous factors that cause the infection because of its wide spread spectrum, because of the range of infection from simple to serious, as these bacteria have the potential to spread the infection widely and its toxic effect[(2–4)] .

The aim of the research is to determine the extent of the immune response against staphylococcus antigens in local rabbits.

Material & Methods

Sampling and bacterial isolation:

Swab samples were collected from inflamed wounds in animals and then grown on three culture transplant and it's:

Nutrient Agar

Blood Agar

MacConky Agar

Then placed in the incubator for 24 hours with a temperature of 37.2 c0 and then observed the growth of bacterial colonies on the implant circles, the eye was taken from the colonies and dyed with gram stain and upon examination it was found positive for this dye and it is a cluster bacteria (cocci).

To find out the sex of these bacteria, he took his eye and grew up on another medium, Mannitol Salt Agar, a

special medium for the growth of Staphylococcus, placed in the incubator with a temperature of 37.5 c0 for 24-48 hours observed the growth of bacterial colonies. He then attended a liquid transplant, Brain Heart Infusion Broth, for mitigation purposes. After preparing the medium we planted in one of the tubes bacteria Staphylococcus and then put in the incubator and after 24 hours observed the change of the middle from net to turbidity indicating the growth of bacteria then attended 6 tubes of the liquid implant center in each tube 9ml to perform the mitigation process. He took 1 ml of the tube containing on a bacterium and then put in the tube number 1 and then 1 ml of tube 1 and put in the tube number 2 and then 1 ml of number 2 and put in number 3…. Etc. To tube 6 where we took 1 ml and we neglected it. Then he took from each of the tubes (4-5-6) one ml (1 ml) and the other 0.1ml and put in the dishes of the implant and then poured the center of the implant on top of it and then we moved each dish 25 turns clockwise and 25 cycle counterclockwise for the purpose of mixing bacteria with the culture media. Incubated the dishes were heated at a temperature of 37.5 c0 and for 24-48 hours after which we found the growth of bacteria in the implant editing circles where the growth in the two concentrations for tubes 4-5 was very dense (colonies cannot be counted) as well as for the concentration of 1ml for tube 6. As for the concentration of 0.1ml for tube 6, the number of colonies was 242 colonies by a growing bacteriological on the plate (the colonies were calculated by the colony counting device), by equation. (Number of colonies * inverted dilution * solution size).

When calculating concentration at 1 ml, it was found to be =242*107 colony by bacteria colony/ml. Then killed the bacteria by putting them in a water bath temperature of 100 c0for half an hour, then they took sample and grew on the culture media to make sure that the bacteria were killed and after the incubation for 24 hours /37.5 c0 did not grow the bacteria[5] .

2- Injection of dead bacteria into rabbits:

After killing the bacteria, take three groups of local rabbits in the animal field of the Faculty college of Veterinary Medicine in Fallujah university, took each group of three rabbits that injected the first group with a concentration of 0.5 ml subcutaneous, and injected the second group with a concentration 0.75 ml with the muscle and left the third group for control. After 14 days of the first injection, it was injected with the same concentrations again and a week later the blood was drawn for work immunological experiments.

3- Immunological tests

A- Hypersensitivity Test

Two groups of local rabbits belonging to the Faculty of Veterinary Medicine in Fallujah took each group of thirteen rabbits injected the first group with staphylococcus bacterium antigens killed by heat with a concentration of 0.1 ml inside the skin, and left the second group to control. The thickness of the skin and the diameter of the red circle were measured in the infected group and in the control group after 24-48-72 hours of injection.

B- Isolation of polymorph nuclear neutrophils cell from the blood.

Blood was drawn from the group of infected people and from the control group from the heart by a wine syringe and placed in sterile plastic tubes and sealed container on heparin with a concentration of 500 global units to prevent coagulation and dextran solution to degenerative red blood cells and then gently blended the contents of the tube. The tubes were then placed in the incubator warmly 37 c0 for 45 minutes, after which the plasma layer containing the white blood cells was withdrawn and transferred to another sterile plastic tube. White blood cells were washed with hanks local balanced solution twice by discarding them at 1500 rpm to remove plasma and heparin. The cells were then suspended in the same solution, with a concentration of 1*106 cells/ml[6].

A- The effect of staphylococcus on the reduction of Nitroblue tetrazolium stain (NBT for PMNs cells).

Put 0.75ml of PMNs cell stuck in silicone-coated glass tubes and then add 0.75ml of NBT dye prepared according to the method[7] the tubes calmly spin the hand to mix the contents of the tube with the provisions of the provisions placed the pipes then in the incubator temperature 37c0 for 25 minutes, After the end of the incubation period he took 20 microliters from each tube to a glass slide and spread gently and then left to dry and fixed with methanol and then dyed your dye as a

muzzle prepared according to the method of Allen and his group[8] for 15 minutes after which 200 cells under the optical microscope 100 X were calculated for the purpose of extracting the percentage of cells that were able to produce super oxide ion and thus reduce the yellow dye NBT to the blue furor granules that deposit in the eye-cell[7]. After the end of the incubation period he took 20 microliters from each tube to a glass slide and spread gently and then left to dry and fixed with methanol and then dyed by Geimsa stain prepared according to the method of Allen and his group[8] for 15 minutes after which 200 cells under the optical microscope 100 X were calculated for the purpose of extracting the percentage of cells that were able to produce super oxide ion and thus reduce the yellow dye NBT to the blue furor granules that deposit in the eye-cell[7] .

A- The effect of Staphylococcus infection on the heat-dead candida yeast:

· Preparation of AB blood group

Pull 10 ml of the blood of a healthy people AB and put it in a sterile plastic tube and leave at room temperature for 30 minutes and then centrifuge quickly 2000 cycle/ minute for 15 minutes, pull the top layer containing the required material (antibodies and complement), and save at a temperature -20c0 until use.

· Phagocytic examination

Placed in sterile plastic tubes 0.25 ml of PMNs cell that concentration 1*10 6 cells/ml added 0.25ml of heat-killed yeast cells prepared by method (Wilkinson, (1977), by 1 cell PBNs: 4 killed yeast cells It was added 0.25ml of AB human blood group, after which the tubes were incubated at a temperature of 5-10% Co2 for periods (30-60-90-120) minutes where they prepare a tube for all time and for all trials (Harry etal., 2014). Then I made four slides for each tube left to dry and fix methanol for two minutes and then dyed with a pigment giemza for 15 minutes calculate 200 phagocytic cells and not phagocytic cells using optical microscope zoom 100 X to calculate the pharynx coefficient =

Pharyngeal coefficient =number of pharyngeal cells/total pharyngeal cell number (phagocytic cells + not phagocytic cells) x 100[6] .

Results

Table 1 shows late hypersensitivity reactions when staphylococcus antigens are injected into the skin and have the highest value for skin foldfish when heat-killing antigens are injected at 0.1 ml in the skin 9.50 mm at a time of 48 hours compared to control 1.48 mm, and the highest red diameter rate was 3.54 mm at a time of 24 hours compared to control (zero).

Table 1: shows late hypersensitivity reactions when injecting heat-killing antigens to Staphylococcus bacterium with a concentration of 0.1ml in the skin.

R	CONTROL	R	CONTROL	
رارحالا رطق		دلجلا كمس		
3.54 0.89± A	0 B	8.60 1.15± A	1.53 0.14± B	24hr
3.26 0.83± A	0 B	9.50 0.92± A	1.48 0.13± B	48hr
2.41 0.19± A	0 B	6.17 0.83± A	1.54 0.15± B	72hr

Different capital letters indicate that there are significances differences between different times below the level ($p<0.05$).

The results showed that the highest level of PBNs cell activities on the candida yeast aunt in the injury was 81.30% when the concentration was injected 0.75ml into the muscle compared to control 79.33%, although the lowest value of the candida yeast aunt was recorded 65.50% compared to control 68.66% and there were significances differences at this time, show table 2.

Table 2: Shows the effect of injecting heat-killing Staphylococcus antigens with a concentration of 0.75ml in the muscle on the candida yeast aunt.

120	90	60	30 min	
64.66 3.84± B	79.33 2.33± A	78.66 3.38± A	68.66 2.33± B	CONTROL
71.20 1.46± B	81.30 0.23± A	77.80 1.00± A	65.50 2.11± C	TREATED

Different capital letters indicate that there are significances differences between different times below the level ($p<0.05$).

While the highest value in the candida yeast aunt at the injection concentration of 0.5ml under the skin was 79% compared to the control of 75.33%, and the percentage of the aunt of the Candida yeast was 66.33% compared to the control 68.66%. Show table 3.

Table 3: shows the effect of injecting heat-killing Staphylococcus antigens with a concentration of 0.5ml under the skin on the candida yeast aunt.

	30 min	**60**	**90**	**120**
CONTROL	64.66 3.84± A	79.33 2.33± B	75.33 6.69± AB	68.66 2.33± AB
TREATED	69.73 0.88± B	73.83 2.37± AB	79.00 2.08± A	66.33 4.40± B

Different capital letters indicate that there are significances differences between different times below the level ($p<0.05$).

As for the reduction of NBT dye by PMNs cells, the highest percentage was 14.70% when injecting the antigen at a concentration of 0.75 ml in the muscle compared to control 10.63%, while the reduction of NBT dye was 12.43% when injecting the antigen at a concentration of 0.5 ml under the skin, Show table 4.

Table 4: shows the effect of injecting heat-killing Staphylococcus antigens with a concentration of 0.75ml in the muscle and 0.5ml subcutaneous concentration on the reduction of NBT pigment.

IM	SC	CONTROL	
14.70 0.52± A	12.43 0.46± B	10.63 0.65± B	NBT

Different capital letters indicate that there are significances differences between different times below the level ($p<0.05$)

Discussion

The results of the study showed that the highest diameter of the redness circuit was 3.54 mm while the highest rate of skin fold thickness was 9.5 mm when injecting 0.1 ml of staphylococcus spp antigens. In the skin at the time 24 and 48 hours in respectively and then start the area of the irritated area and the thickness of the skin folds gradually decreased and there were significances differences between the transactions and its control. These results are consistent with his findings[9] When injecting antigens of the weakened pseudomonas aeruginosa bacteria as well as consistent with the study[9] On the heat-weakening E. coli bacteria, they found that the injection of antigens of these bacteria led to the significances rise in both the thickness of the skin and the area of the irritated area in the local rabbits to 3.21 mm and 4.25 mm after 24 hours of antigen injection respectively. Table 2 shows the susceptibility of PMNs cells to the aunt of candida yeast in the vitro, as the results showed that the highest level of the aunt of the candida yeast aunt in the totals of transactions injected with antigen 81.3% compared to control 79.33% and there were no significant differences between the injected groups and control groups. As for the reduction of the dye of Nitro blue tetrazolium (NBT), the results showed that there are significant differences in the injection of antigens compared to non-treated control and the highest reduction rate of 14.70% when injecting antigens at a concentration of 0.75 ml in the muscle compared to control 10.6% these results are consistent with the findings[10] which stated that the rates of pharyngeal coefficient in mice treated with polysaccharide lipid for klebsiella pneumoniae show difference significant compared with the control mice's. In 2002 Cortes et al explained the high rates of pharyngeal coefficient in mice treated with polysaccharides fatty to klebsiella pneumoniae bacterium due to the ability of these antigens to stimulate the components of the complementary system, especially c5a and c3b, which are involved in the process of opsonization and attracting the neutrophilic cells to the site of the infection respectively[11] .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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