U of K. J. Vet. Med. & Anim. Prod. Vol. 3, No 2, 2012 (127-141)

THE ROLE OF CHOLESTEROL IN ENHANCING GROWTH OF *Staphylococcus aureus*

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المستخلص

أجريت هذه الدراسة التحليلية لتقييم تاثير الكولسترول في تعزيز نمو المكورات العنقوديه التي تسبب التهاب الجلد ، تقييم اثر بدرة الكولسترول بتراكيز مختلفه علي المكورات العنقوديه ولتقييم امكانية معيشة المكورات العنقوديه في امصال البدناء وغير البدناء . تمت تغطية الوسط الزراعي بتراكيز ومستويات مختلفه من بدرة الكولسترول ومصل الكولسترول ، ثم بعد ذلك تم تغطية الوسط الزراعي بمقدار 0.25مل محلول مخفف من المكورات العنقوديه الذهبيه قد اعد مسبقا. تم وضع الوسط الزراعي في الحضانه في درجه37 درجه مئويه لمدة 24 ساعه.هذه الدراسه اوضحت زياده كبيره بين متوسط عدد المستعمرات البكتيريه في مستويات مصل الكولسترول الطبيعي في الدم (الامصال لغير البدناء) ، الكولسترول في خط الحدود(الحد الفاصل) والكولسترول المرتفع (امصال البدناء) . وهناك ايضا فروقات ذات دلاله احصائيه بين متوسط عدد المستعمرات البكتيريه في مستويات امصال البدناء وغير البدناء. اوضحت هذه الدراسه ايضا زياده كبيره بين متوسط عدد المستعمرات البكتيريه في تراكيز مختلفه من بدرة الكولسترول: تراكيزعادية لبدرة الكولسترول وتراكيز بدرة كولسترول في خط الحدود و ذات تركيز عالي . اظهرت الدراسة ان هناك فروقات ذات دلاله احصائيه بين متوسط عدد المستعمرات البكتيريه في مستويات امصال الكولسترول و تراكيز بدرة الكولسترول.

كما تبين وجود ارتباط بين مستويات الكولسترول في الدم ومدة نمو المكورات العنقوديه الذهبيه. هذه النتائج تشير إلى أن ارتفاع مستويات الكولسترول في الدم يمكن أن تسهم في انتشارالمكورات العنقودية،وخاصة المكورات العنقوديه الذهبيه.

Abstract

This is a prospective analytical case control study that used to assess the effect of cholesterol in enhancing growth of *Staphylococcus* ,the effect of different concentrations of cholesterol powder on growth of *Staphylococcus* and the survival of *Staphylococcus* in obese and non obese sera.

Nutrient Agar (NA) was spread with different concentrations of cholesterol powder and cholesterol sera. Agar plates without cholesterol were prepared and used as controls. *S.aureus* at 10^{-7} cfu/ml was used at 0.25 ml to cover each plate and was spread by glass rod, then incubated aerobically at 37^{o}C for 24 hours. Numbers of colonies on each plate were counted by digital colony counter.

In this study significant differences were recorded between mean colony counts of normal cholesterol sera (non-obese sera), border line high and high cholesterol sera levels (obese sera). Also there was a significant difference between mean colony count in obese and non-obese sera levels.

Significant differences were seen between mean colony counts of normal cholesterol powder, border line high cholesterol powder and high cholesterol powder Concentrations. The differences between different concentrations of cholesterol powder on the effect growth of *S.aureus* were found significant.

The differences between mean colony counts in different levels of cholesterol sera and different concentrations of cholesterol powder were significant.

A relation between the levels of serum cholesterol and the duration of growth of *S. aureus* was noticed.

These results proved that the presence of high cholesterol levels in serum may contribute to the proliferation of *S.aureus*.

Introduction

Staphylococcus is a genus of family Micrococcaceae. *Staphylococcus* (from the Greek *'Staphyle'*, bunch of grapes) owes it's name to the fact that it appears to be clustered as a result of division (Gabrial and Virella, 1997).

Staphylococci are a common type of bacteria that live on the skin and mucous membranes (eg. in nose) of humans.

Skin infections are the most common type of disease produced by *Staphylococcus*. Staph infections of the skin can progress to impetigo or

cellulitis . In rare cases, a serious complication known as scalded skin syndrome can develop. In breastfeeding women, *S.aureus* can result in mammitis (inflammation of the breast) or in abscess of the breast. In infants *S.aureus* infection can cause a severe disease called staphylococcal scalded skin syndrome (SSSS) (Curran and Al-Salihi, 1980); a severe form of this is Ritter's disease seen in neonates.

Curran and Al-Salihi, (1980) stated that *S.aureus* can infect other tissues when barriers have been breached (e.g., skin or mucosal lining). This leads to furuncles (boils) and carbuncles.
Cholesterol is a waxy steroid metabolite found in the cell membranes and transported in the blood plasma of all animals (Emma Leah, 2009). It is an essential structural component of mammalian cell membranes, where it is required to establish proper membrane permeability and fluidity. In addition, cholesterol is an important component for the manufacture of bile acids, steroid hormones, and fat-soluble vitamins.

Although cholesterol is an important and necessary molecule for mammals, a high level of serum cholesterol (Hypercholesterolemia) is an indicator for diseases such as heart disease, kidney disease and high blood pressure (especially in those who are suffering from obesity).

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, (WHO, 2000; Haslam and James, 2005). Obesity is a risk factor for several serious disease states such as, diabetes, heart disease, stroke, hypertension and some type of cancer. The connection of obesity with skin and soft tissue is less well studied than with other disease states. This relationship has not garnered as much attention as other disease states related to obesity, but the current research demonstrates that there is relationship between the two (Juliana Swiney, 2010).

Wolf and Proceed, (2008) found that people who are overweight or obese have an 8% greater prevalence of skin condition symptoms than the person who has normal weight.

Studies on effect of cholesterol in stimulating *S.aureus* growth showed that in some chronic blepharitis disease groups certain *Staphylococcus* species were capable of hydrolyzing cholesterol esters; the authors tested the hypothesis that the resulting cholesterol might affect growth of *Staphylococcus aureus*. The results of this study suggest that the presence and hydrolysis of cholesterol esters of meibomian secretions may contribute to the proliferation of *Staphylococcus* spp, especially *Staphylococcus aureus*, observed in some chronic blepharitis disease groups (Shine *et al.,* 1993).

The aim of this study was to assess impact of high cholesterol sera of obese subjects in enhancing growth of *Staphylococcus* comparing with cholesterol of non obese ones, to detect effect of different concentrations of cholesterol powder on growth of *Staphylococcus* and to assess the survival of *Staphylococcus* in obese and non obese sera.

Materials and Methods

The study population included **30** of human subjects who were obese and **24** non- obese used as negative controls.

Isolation of *Staphylococcus aureus*:

Staphylococcus aureus strains were isolated from abscesses of patients attended at Khartoum hospital using sterile swabs. The swabs were first soaked in sterile normal saline before they were used to scratch the tunica of the abscess. Swabs were kept refrigerated and transmitted as quickly as possible to the laboratory. There, swabs were cultured on Nutrient Agar prepared freshly and stored at 4^{o}C. Cultured plates were incubated at 37^{o}C overnight. Cultures were examined for growth and purified by further sub culturing. Cultures showing Gram positive cocci arranged in groups were further identified according to the method of Barrow and Feltham (2003), catalase tests were made,to gether with coagulase test, both as slides and tubes, using human plasmas. Coagulase positive isolated, and identified as *S.aureus* were used for this study. Strains of coagulase positive isolates were stored in a slope of Nutrient Agar and preserved in the refrigerator.

- Cholesterol powder used was obtained from **(**sd FiNE-CHEM LiMiTED)

Test of sera for cholesterol level:

Three tubes with one ml of working reagent were prepared to serve as standard (STD), test and blank . Ten µL of sample and standard were added to the STD and the test tubes, then mixed and incubated for ten minutes. The absorbance (A) of samples and standard were read, against the blank.

Effect of sera of obese and non- obese persons on growth of *Staphylococcus aureus*:

Nutrient broth was inoculated with culture of *S.aureus* grown on Nutrient Agar slope, then mixed and incubated at 37^{o}C for 3 hours. One ml of the three hours culture was removed and mixed with 9ml of normal saline for 10 fold serial dilutions.

Half ml of sera of obese persons and 0.5ml of sera of non obese persons (by automatic pipette) were used to cover the plates of nutrient agar by using glass rod. Plates were left for ten minutes to dry. Predetermined serially diluted cultures of *S.aureus* at dilution 10^{-7} were used as 0.25 ml to cover each plate and spread by glass rod. The cultured plates containing obese sera and non- obese sera were then incubated aerobically at 37^{o}C for 24 hours. Numbers of colonies on each plate of obese and non- obese sera were counted by a digital colony counter.

Effect of cholesterol powder with different concentrations on growth of *Staphylococcus aureus:*

Different concentrations of cholesterol powder were chosen to simulate those of human concentration.

Different concentrations of sterile cholesterol powder 70, 100, 168, 195, 200, 220, 250, 350, 450, 550 and 1000 mg were placed on sterile plates then sterile Nutrient Agar was poured on plates, mixed well and were left to solidify.

Predetermined serially diluted culture of *S.aureus* at dilution 10^{-7} were used as 0.25ml and spread over each plate. The cultured plates were then incubated at 37^{o}C for 24 hours. Numbers of colonies were counted by a Digital colony counter.

Survival of *Staphylococcus aureus* in sera of obese and non- obese persons:

One ml of serum of obese and non- obese persons and one ml of normal saline as control were each inoculated, using two drops of an overnight culture of *S.aureus*. The tubes were incubated at 37^{o}C for 4, 6 and overnight hours. After 4 and 6 hours one drop from each culture of obese and non-obese sera and control were used to cover Nutrient Agar plates and spread by a glass rod. The cultured plates were then incubated at 37^{o}C for 24 hours. Growth of colonies was observed. The tubes were then stored in a refrigerator (4^{o}C) for an overnight, after which they were sub- cultured in Nutrient Agar.

Results

Figure 1 shows a significant increase between mean colony counts of normal cholesterol sera (non-obese sera), border line high cholesterol sera and high cholesterol sera (obese sera) levels.

Normal cholesterol sera showed a mean 7.09×10^{7}cfu /ml, P.value < 0.005, border -line high cholesterol sera mean was 1.273×10^{8}cfu /ml, P.value < 0.000 highly significant while high cholesterol sera showed a mean: 2.294×10^{8}cfu /ml, P.value < 0.004.

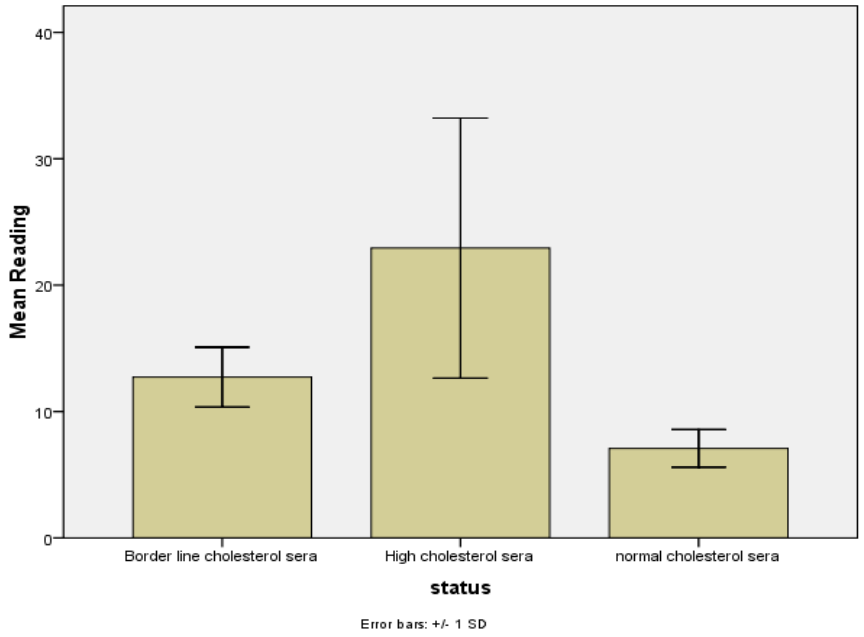

Figure (1): The mean colony count of border- line high cholesterol sera, high cholesterol sera and normal cholesterol sera levels.

Figure 2 shows the significant comparison between mean of colony count in high cholesterol sera (obese sera) and normal cholesterol sera (non obese sera).
(Mean): (2.294×10^{8} cfu /ml versus 7.09×10^{7} cfu /ml), $P < 0.003$

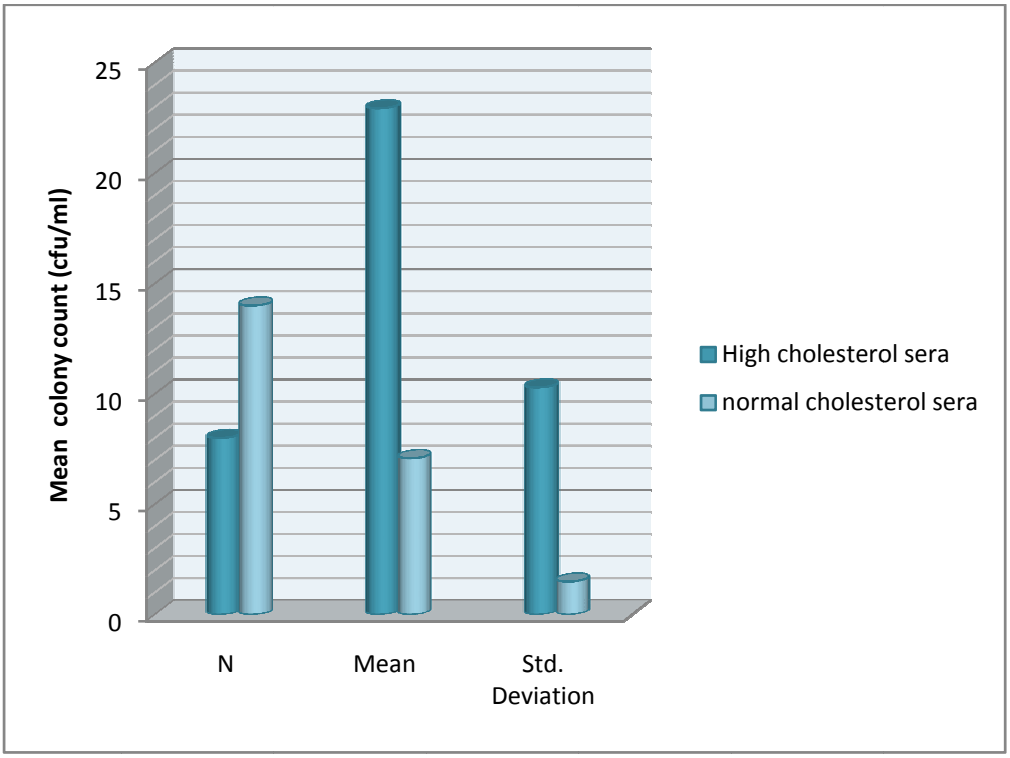

Figure (2): Comparison between mean colony counts of high cholesterol sera and normal cholesterol sera levels.

Figure 3 shows significant differences between mean of colony count of normal cholesterol powder, border- line high cholesterol powder and high cholesterol powder concentrations.
Normal cholesterol powder concentration showed a mean of 9.4×10^{7}cfu /ml, $P < 0.179$, border- line high cholesterol powder showed a mean of 1.30×10^{8}cfu /ml, $P < 0.014$ while high cholesterol powder had mean of 2.13×10^{8}cfu /ml, $P < 0.007$

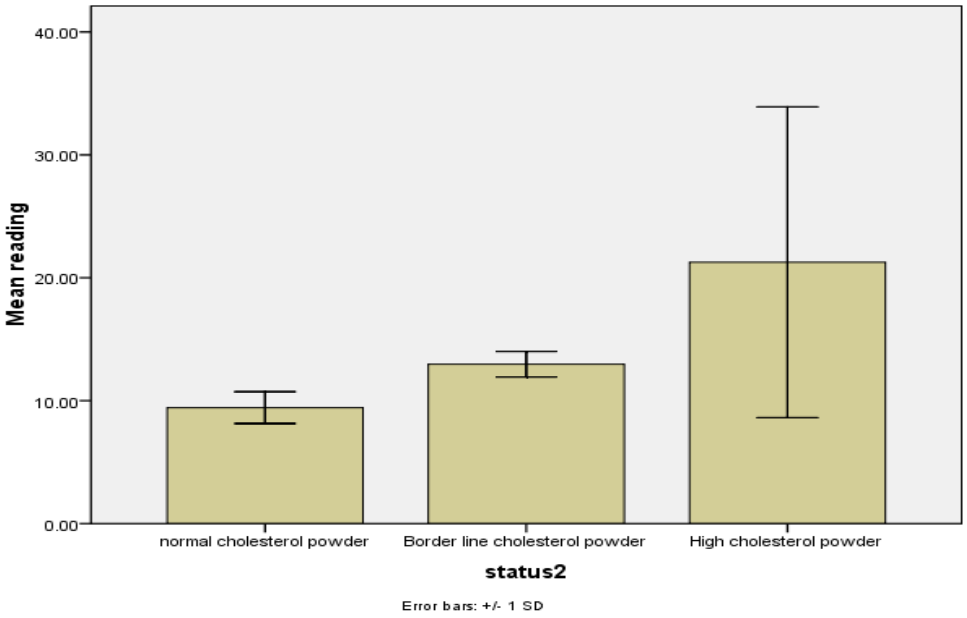

Figure (3): Mean colony count in normal, border- line high and high cholesterol powder concentrations.

Figure 4 shows comparison between mean of colony count in high cholesterol powder and normal cholesterol powder.

Normal cholesterol powder had a mean of 9.4×10^{7}cfu /ml, high cholesterol powder had a mean of 2.13×10^{8}cfu /ml. ($P < 0.004$)

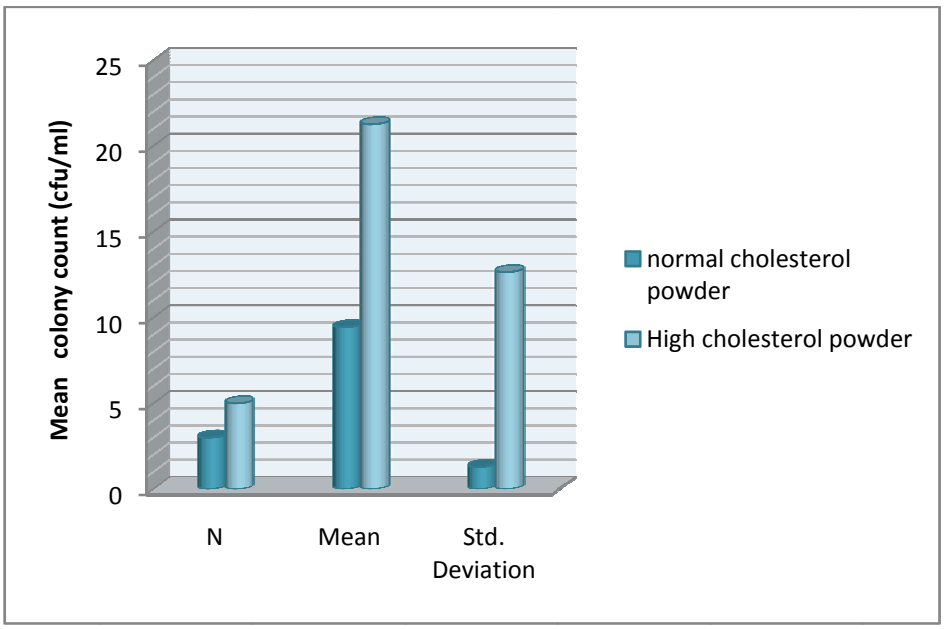

Figure (4): Mean colony count in high cholesterol powder and normal cholesterol powder concentrations.

Figure 5 shows the relation between the levels of serum cholesterol and the duration of growth of *S.aureus.*

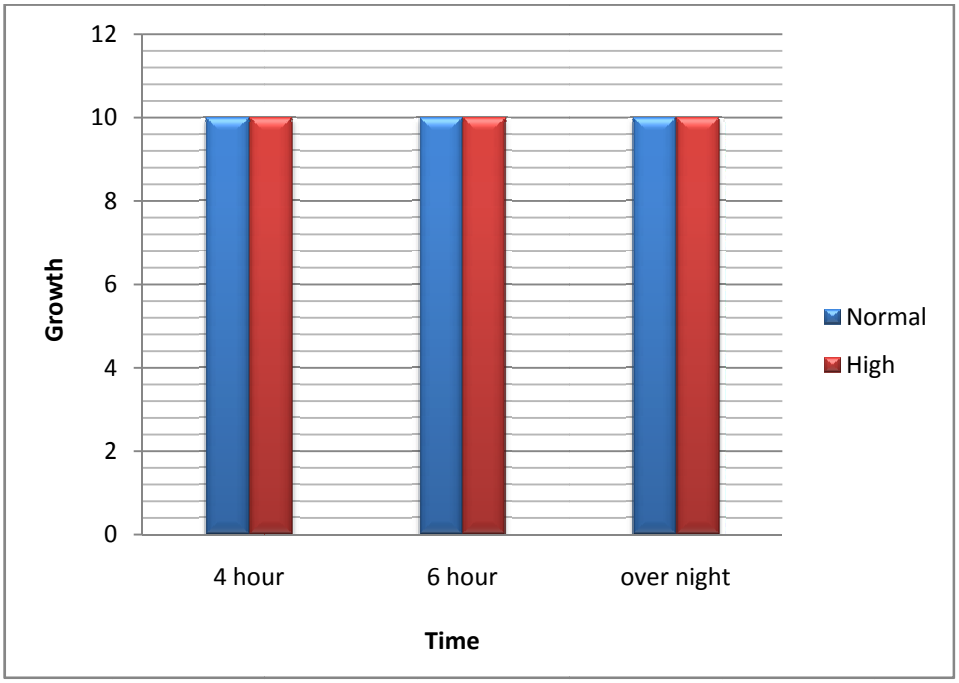

Figure (5): Relation between the duration of *S.aureus* growth and levels (normal and high) of serum cholesterol.

Figure 6 shows significant comparison between means of colony counts in different levels of cholesterol sera and different concentrations of cholesterol powder.

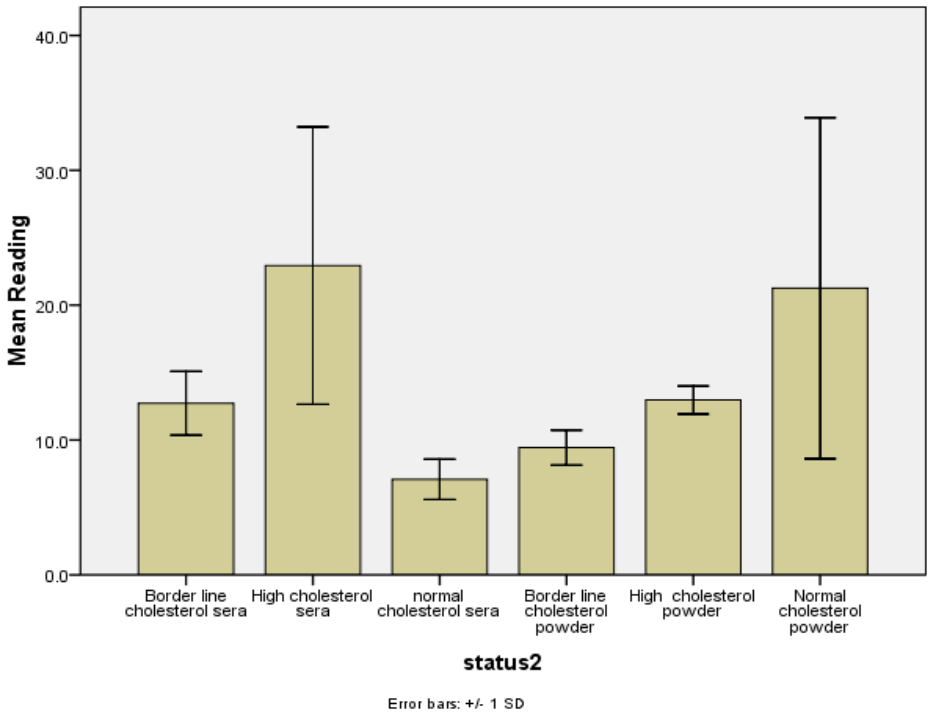

Figure (6): Comparison between means of colony counts of *S.aureus* in different levels of cholesterol sera and different concentrations of cholesterol powder.

Discussion

This study was conducted to see the effect of cholesterol on growth and survival of *S.aureus* which is main causative agent of skin infection.

The result of this study showed significant increases between mean colony count in different levels of normal cholesterol sera, border- line high cholesterol sera and high cholesterol sera in obese and non- obese persons.

This finding agrees with Hassan (1996) who demonstrated that sera collected from fattened animals (sheep) supported a better growth of *S.aureus* subsp *anaerobius* than sera collected from non-fattened animals.

This finding also agrees with Shine *et al.,* (1993) who reported similar results of effect of cholesterol in stimulating *S.aureus* growth. Their study showed that in some chronic blepharitis disease groups certain *Staphylococcus* species were capable of hydrolyzing cholesterol esters. *S.aureus* growth as stimulated in Mueller-Hinton broth by cholesterol was determined by colony forming units. Growth stimulation by cholesterol and other additives were also determined by the optical density at 650 nm method. Shine *et al* (1993) proved that cholesterol stimulated *Staphylococcus aureus* growth significantly during the first 24 hr period and for the total 48 hr period when compared with the respective control. These results suggest that the presence and hydrolysis of cholesterol esters of meibomian secretions may contribute to the proliferation of *Staphylococcus* spp, .

In contrast, Noble (1990) attempted to discern a relationship between skin flora and free fatty acids in man. Fatty acids have often been included amongst the factors thought likely to suppress organisms on skin.

The present finding disagrees with Jay-Naidoo (1980) who mentioned that cholesterol alone had no significant effect on the growth of *S.aureus* at either 5.5 or 7pH. However, when cholesterol (10mg /ml) was added to medium containing linolic acid (4mg /ml), the inhibitory effect of the fatty acid was reduced, but at pH 5.5 cholesterol plays no advantageous effect against inhibitory action of linolic acid. The various cholesterol esters had no effect on growth of sensitive and resistant variants at either pH. 5.5 or7 values.

The present study demonstrates the relationship between serum cholesterol levels (obesity) and growth of *S.aureus* which causes skin infection (abscess). This relation was explained previously by Bukhet (2005) who reported the correlation between abscesses size (cm^2) and body weight (kg) of fattened and unfattened sheep where abscess size increased after infection proportionally to the body weight.

Our study agrees with Wolf and Proceed (2008) who found that people who are overweight or obese have an 8% of greater prevalence of self-reported skin condition symptoms than the person who has normal weight.

This study also demonstrated a significant increase when comparing the mean colony count of cholesterol powder simulating that found in human sera as well as border- line high and high cholesterol. Also there is a significant comparison between different concentrations of cholesterol powder. No relevant literature could be traced on the effect of cholesterol powder on growth of *Staphylococcus* in and outside Sudan.

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