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BACTERIAL ISOLATED AND DIAGNOSIS FROM THE RESPIRATORY SYSTEM OF SMOKERS AND NON SMOKERS INDIVIDUAL AND COMPARE THE BACTERIA WITH THE ISOLATED BACTERIA FROM SOME TYPES OF CIGARETTES WHICH COLLECTED FROM THE MARKETS AND MEASURES THE RELATIONSHIP OF HEMOGLOBIN AND BLOOD VISCOSITY IN BOTH TYPE OF INDIVIDUALS

Zeayd Fadhil Saeed

* Foundation of Technical Education, Technical Institute of Samawa, Nursing Department
Email: zeaydfadhil@gmail.com

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Abstract

Fifty sample was collected and divided into three groups, the first twenty (20) of them collected from the respiratory system of smokers individual, and the second twenty (20) samples was collected from the respiratory system of non smokers individual, and the latest group collected from ten types (10 type) of cigarettes which were used in markets by smokers in Al Muthana City in Iraq.

The first and second of the collected samples groups had been collect by coughing on sterile Petri dishes with nutrient agar and then incubated in 37C° for 24 hour to see the colonies growth of bacteria to isolate and diagnosis. In addition for that we collected a blood samples from these groups to measures the differentiations in hemoglobin range and blood viscosity in both groups individuals.

On the third groups of samples which are ten samples of cigarettes (10 types of cigarettes) which collect from the markets which were (**Aspin, Royal, Oscar, Pine, Blue Bon, White Bon, Master**, **Marlboro, Gauloises , kent**) which the tobacco of each type has been collected in separate test tube and soaked with distal water for 24 hour and then taken 1 ml of the clear liquid to culture it on sterile Petri dishes with nutrient agar and then incubated in 37C° for 24 hour to see the colonies growth of bacteria to isolate and diagnosis.

We detect that the first group of samples (smokers individual) were infect with a dense growth of bacterial colonies of *Staphylococcus aureus* and *Staphylococcus albus* which may reach to 80% of the summation collected samples which may cause abscess and necrotic pneumonia and may be lead to death. Also we detected there are *Clostridium perfringens* and *Clostridium botulinum* bacteria with 55% of the collected samples these bacteria may be lead to gas gangrene in pulmonary tissue. In addition we detected there are *Streptococcus pyogen* which reach to 35% of the collected samples these bacteria may cause septic inflammations.

So the second group of sample appear that there are colonies growth of *Staphylococcus supp.* With 65%, *Streptococcus supp.* With 10% , *Clostridium supp.*

15% and *fungus* with 80% of the collected sample.

Also there are many types of bacteria which isolated and diagnosed from ten types of cigarettes(**Aspin, Royal, Oscar, Pine, Blue Bon, White Bon, Master**, **Marlboro, Gauloises , kent**) which for use smoking in Al- Samawa city of Iraq. So the culture of Drenched tobacco of each type of cigarette showed that there are *Clostridium supp.* 100% , *Staphylococcus supp.* With 57% , spores 71%, and fungus 14% of the collected sample.

So the blood sample of the of smoker group showed that there are an Inverse relationship and positive relationship between the hemoglobin and blood viscosity with smoker age respectively of the smoker individual that is mean the Hb concentration decrease and blood viscosity increase with smoker age progress. On the other side the blood sample of non smoker group showed that there are positive relationship between the hemoglobin and blood viscosity with the non smoker age progress.

Introduction

Smoking is a practice in which a substance is burned and the resulting smoke breathed in to be tasted or inhaled. Most commonly the substance is the dried leaves of the tobacco plant which has been rolled into rice paper into a small, round cylinder called a "cigarette". This is primarily practiced as a route of administration for what has come to be termed "recreational drug use" because the combustion of the dried plant leaves releases active substances into the body. In the case of cigarette smoking these substances are contained in a mixture of aerosol particles and gasses and include the pharmacologically active alkaloid nicotine; the vaporization creates heated aerosol and gas to form that allows inhalation and deep penetration into the lungs where absorption into the bloodstream of the active substances occurs. [1]

Cigarettes are primarily industrially manufactured but also can be hand-rolled from loose tobacco and rolling paper. Other smoking implements include pipes, cigars, bidis, hookahs, vaporizers, and bongs. It has been suggested that smoking-related disease kills one half of all long term smokers but these diseases may also be contracted by non-smokers. A 2007 report states that, each year, about 4.9 million people worldwide die as a result of smoking.[2]
Perception surrounding smoking has varied over time and from one place to another; holy and sinful, sophisticated and vulgar, a panacea and deadly health hazard. Only relatively recently, and primarily in industrialized Western countries, has smoking come to be viewed in a decidedly negative light. Today medical studies have proven that smoking tobacco is among the leading causes of many diseases such as lung cancer, heart attacks, COPD, erectile dysfunction, and can also lead to birth defects. [3]

Blood viscosity is a measure of the resistance of blood to flow. It can also be described as the thickness and stickiness of blood. This biophysical property makes it a critical determinant of friction against the vessel walls, the rate of venous return, the work required for the heart to pump blood, and how much oxygen is transported to tissues and organs. These functions of the cardiovascular system are directly related to vascular resistance, preload, afterload, and perfusion, respectively[4].

Hemoglobin (**Hg**) is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates[5] (with the exception of the fish family Channichthyidae[6]) as well as the tissues of some invertebrates. Hemoglobin in the blood carries oxygen from the respiratory organs (lungs or gills) to the rest of the body (i.e. the tissues) where it releases the oxygen to burn nutrients to provide energy to power the functions of the organism in the process called metabolism.

The goals of this research are known and isolated the bacteria in the respiratory system in the smokers and non smokers individual and diagnosis and isolated the bacteria which grown on the tobacco of some types of cigarettes

which found in the markets today for smoking in Al Muthana city in Iraq, In addition for that measured the hemoglobin concentration and the range of blood viscosity in blood sample of smokers and non smokers individual.

Material and method

The materials which used in this research was :

- Sterile Petri dishes with neutering Agar
- Slides
- Burner
- Loop
- Incubator
- Filter paper
- Gram Stain
- Test tubes
- Hemocue HB 201 microcuvette.
- Microhaematocrit centrifuge.

We tried to collect the 40 people which divided into 2 groups (20 smokers and 20 non smokers) , The two groups were selected to be equal at the age range as the following (15, 20, 22, 24, 27, 30, 31, 33, 36, 39, 40, 41, 43, 45, 49, 50, 51,52, 54, 60) years of old.

For that we can divided the methods into two divisions, firstly the methods of collecting the cough sample from smokers and non smokers individuals, and the second divisions include the collection of blood sample of smokers and non smokers to measured the hemoglobin concentration and blood viscosity.

A. Cough sample collection.

First of all we must prepare 1000 ml of nutrient agar by mixing 28 mg of nutrient agar powder with 1000 ml of distal water in flask and then but the mixture in the autoclave for sterilization under temperature 121 C° for 15 minute. After complete the media sterilization must prepare and pour the media in (53) of Petri dish for collecting the cough sample from the (20) smoker individual and (20) non smoker individual and 10 sample for culturing the Drenched tobacco and (1) Petri dishes must be control for each group.

First group of smokers people in laboratory must make strong cough on sterile Petri dish to adhere the bacteria of their respiratory system on the media and incubation in the incubator in 37 C° for 24 hour, and by that will be 20 Petri dishes contaminated with cough of 20 smokers person.

Second group of non smokers people in laboratory must make strong cough on sterile Petri dish to adhere the bacteria of their respiratory system on the media and incubation in the incubator in 37 C° for 24 hour, and by that will be 20 Petri dishes contaminated with cough of 20 non smokers person.

Also we collect 10 type of cigarettes which are available in the markets for smoking which are (**Aspin, Royal, Oscar, Pine, Blue Bon, White Bon, Master**, **Marlboro, Gauloises , kent**). So in the laboratory we collect the tobacco of each type of cigarette in separated test tube with mixed of distal water 24 hour to be Drenched tobacco and then we take 1 ml of the clear fluid of Drenched tobacco from each tube for culturing on the nutrient agar separately, and then put the Petri dish of each type of Drenched tobacco sample in incubator with 37 C° for 24 hour for bacterial growth and diagnosis by detected the grown bacteria by staining with Gram stain.

B. Blood sample collection:-

We collect 40 blood sample of two groups (20 smoker and 20 non smokers) for measuring the hemoglobin concentration and blood viscosity for each group and compare between the results.

1. **Method of hemoglobin concentration detection**
    - Wear the disposable gloves throughout the procedure and Prepare our equipment. Unwrap a band-aid and lay the band-aid, a Hemocue HB 201 microcuvette, a lancet and cellulose swab on a disposable paper towel.
    - Ask the patient for permission to do the test. Explain why we are doing the test in simple terms and how it will be done. For example, “this test tells us if blood has enough iron in it. and I will take a few drops of blood from the finger. And then We will discuss the results.”

- Select a site for the blood sample, usually the side of the tip of the middle or fourth (ring) finger. If needed, warm the hands or feet by rubbing them together or between your own hands to help improve blood flow.
- Moisten the cellulose swab with alcohol and clean the site to be tested. Let the alcohol evaporate or wipe it with a dry cellulose swab.
- Using our thumb, lightly press the finger from the top of the knuckle toward the tip. This stimulates blood flow toward the sampling point. Hold the finger firmly and quickly puncture the site with the sterile lancet. Drop the used lancet into an impervious (Sharps) container with a tight-fitting lid. This type of container is leak proof and cannot be punctured by sharp objects.
- Wipe away the first two drops of blood. When the third drop is large enough to fill a microcuvette, touch the tip of the microcuvette to the blood to draw the blood up into the microcuvette in one continuous process. Do not refill. And hold a dry cotton ball or cellulose swab on the puncture site for minute or two to stop the bleeding. Place a band-aid on the puncture site.
- Wipe off excess blood from the outside of the microcuvette with a clean, dry cellulose swab, being careful not to touch the open end of the microcuvette. Look for air bubbles in the filled microcuvette. If present, discard the microcuvette and obtain a new sample. Small bubbles around the edge can be ignored.
- Place the filled microcuvette in the cuvette holder and push the cuvette holder in to its measuring position. This must be done within 10 minutes of filling the microcuvette. During the measurement an hourglass with show on the display of the Hemocue machine. The Results are displayed on the Hemocue machine within 15-60 seconds. Record the results on in the client's TWIST file and on their WIC ID card. Interpret the results for the WIC client.
- The normal range of hemoglobin concentration are:- For men 13.5 – 17.5 gram / dL and For women 12.1 – 15.1 gram / dL
-

2. Method of blood viscosity detection

The Blood viscosity is a measure of the resistance of blood to flow. It can also be described as the thickness and stickiness of blood[4]. So we will consider the Hematocrit (Packed Cell Volume – PCV) as an indicator to increase or decrease of blood viscosity, This relationship becomes increasingly sensitive as hematocrit increases. When the hematocrit rises to 60 or 70%, which it often does in polycythemia [7] as the following method:

- Puncture the skin of the finger and collect blood from the capillary directly into heparinized microhaematocrit tube; fill 2/3 of the tube, and Seal one end of the tube with clay or a sealant. Avoid trapping air between the blood and plug.
- Place the tube into a calibrated microhaematocrit centrifuge, sealed ends out against a rubber ring. Place firmly the lid over the centrifuge head. Close the cover. Set the timer (most instruments require 3 to 5 minutes centrifugation time). Centrifuge the tube (usually at 10,000 RPM).
- The tube should be removed and read within a minute or two after the centrifuge has stopped to avoid re-dispersion of cells. Hemolysis should be noted, since this may lower the hematocrit results in relation to the hemoglobin (the hematocrit is 3 times the value of the hemoglobin, if the cells are normocytic).
- Use a lined card, wheel or other device to determine the hematocrit value. They all work by the same principle, measuring the height of the total blood column and the height of the red cell layer.
- **Reference values:** Adult male 38 - 50% , Adult female 36 - 46%.

Result and discussion

After stain the grown bacteria by gram stain and detect the bacteria under microscope the result showed that the samples which collected from smokers there are more growth of *Staphylococcus aureus* and *Staphylococcus albus* which may reach to 80% of the summation collected samples which may cause abscess and necrotic pneumonia and may be lead to death. Also we detected there are *Clostridium perfringens* and *Clostridium botulinum* bacteria with 55% of the collected samples these bacteria may be lead to gas gangrene in pulmonary tissue. In addition we detected there are *Streptococcus pyogen* which reach to 35% of the collected samples these bacteria may cause septic inflammations.

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The result of sample which collected from non smokers showed there are *Staphylococcus supp.* With 65% , *Streptococcus supp.* With 10% , *Clostridium supp.* 15% and *fungus* with 80% of the collected sample. (see the diagram 1)

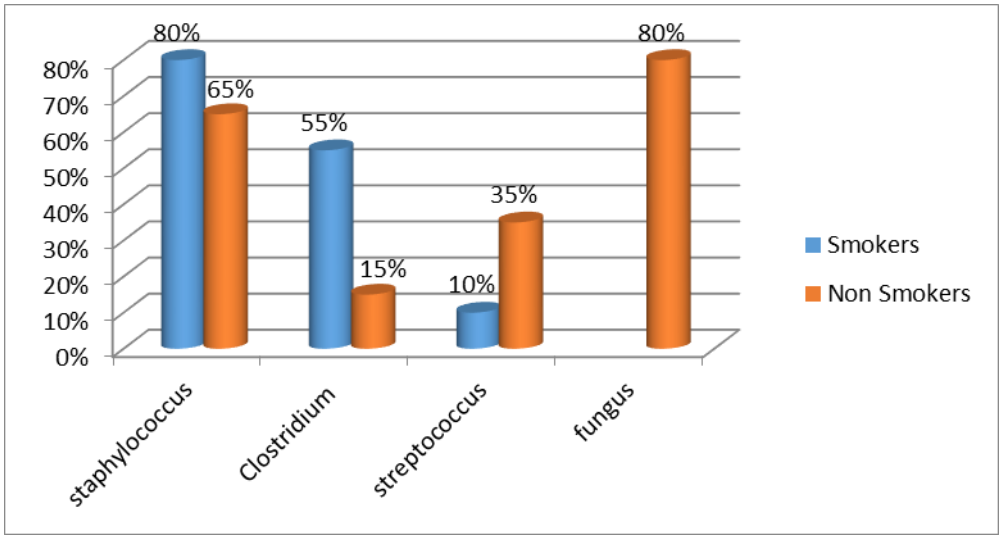

Diagram (1):- Explain a percentage of the isolated bacteria from the respiratory system of smokers and non smokers individual

Also there are many types of bacteria which isolated and diagnosed from ten types of cigarettes(**Aspin, Royal, Oscar, Pine, Blue Bon, White Bon, Master**, **Marlboro, Gauloises , kent**) which for use smoking in Al- Samawa city of Iraq. So the culture of Drenched tobacco of each type of cigarette showed that there are *Clostridium supp.* 100% , *Staphylococcus supp.* With 57% , spores 71%, and fungus 14% of the collected sample. (see the diagram 2)

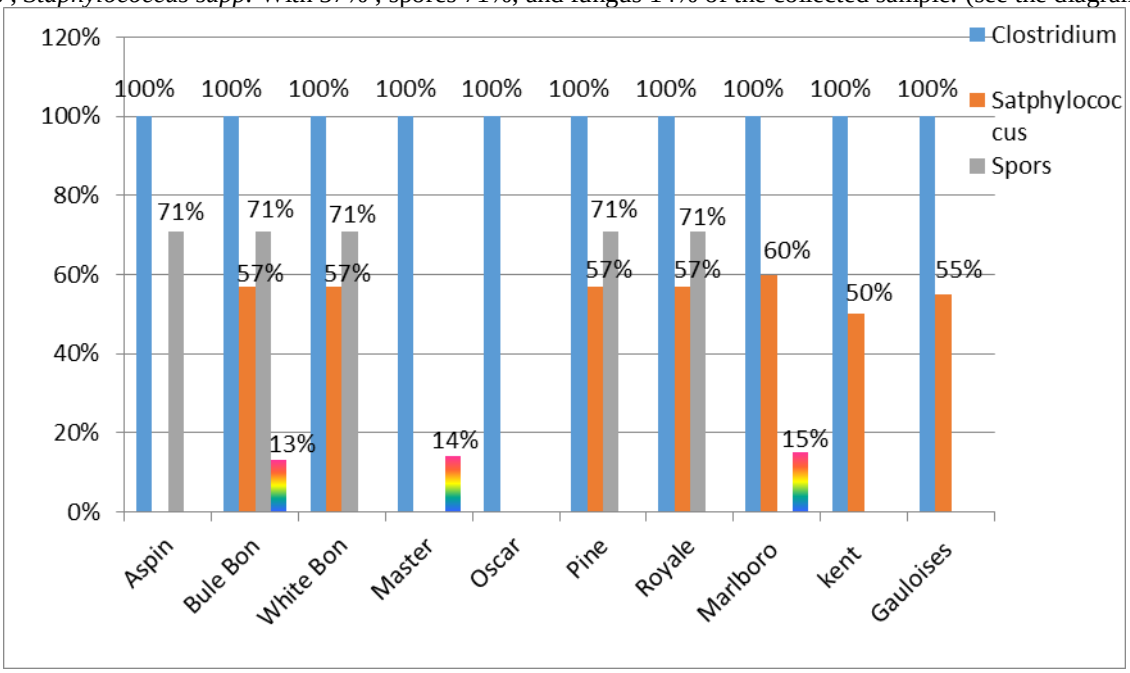

Diagram (2):- Explain the isolated and diagnosed bacteria from 10 types of cigarettes which collected from markets which use smoking in Al- Samawa city of Iraq

So we can see from the diagrams above that there are a good growth with many types of bacteria in the respiratory system of smokers as well as the tobacco of the cigarette consider a suitable environment for bacterial growth.

Also the blood sample analysis explain the relationship between the hemoglobin concentration and the blood viscosity (PCV) with the age of the smokers (we can see the table no 1).

Table (1):- Explain the relationship between the hemoglobin concentration and the blood viscosity (PCV) with the age of the Smokers

Number of Sample	Age With Year	Hemoglobin concentration Gram / dl	PCV Percentage %
1	15	12 G/ dl	41 %
2	20	13.5 G/ dl	41.5 %
3	22	13.3 G/ dl	41.5 %
4	24	13.5 G/ dl	42 %
5	27	12.9 G/ dl	47 %
6	30	12.5 G/ dl	47.5 %
7	31	11.3 G/ dl	47 %
8	33	11.2 G/ dl	47.5 %
9	36	11.3 G/ dl	48 %
10	39	11.1 G/ dl	48.5 %
11	40	11 G/ dl	49 %
12	41	10.8 G/ dl	50 %
13	43	10.5 G/ dl	52 %
14	45	10.5 G/ dl	55 %
15	49	10.4 G/ dl	58 %
16	50	10.4 G/ dl	58 %
17	51	10.3 G/ dl	59 %
18	52	10.3 G/ dl	60 %
19	54	9.5 G/ dl	63 %
20	60	8 G/ dl	65 %

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On the diagram below the date mention that there are an Inverse relationship between the hemoglobin and the age of the smoker individual for that we can see in the diagram no (3) in the 15-20 years old may be the Hb concentration on the normal range, so we can see the Hb concentration directed to decreasment when the age become more years old which may reach to 8 g/dL in 60 years old.

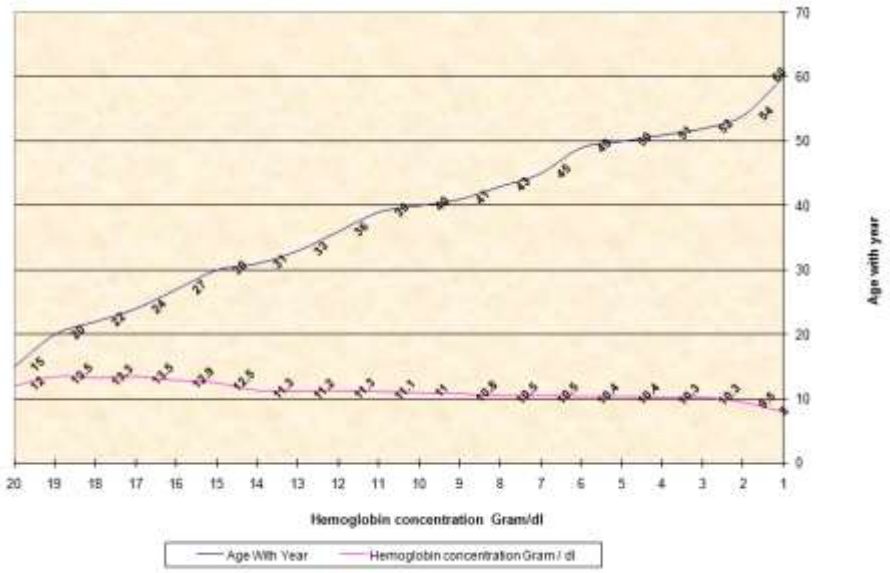

Diagram no 3 : hemoglobin concentration for smokers

Also On the diagram below the date mention that there are an positive relationship between the packed cell volume (PCV %) and the age of the smoker individual for that we can see in the diagram no (4) that the line of PCV% and the age directed on parallel up toward the normal range which are 36-50% , that is mean the viscosity of blood increased when the age become more years old so may be reach to 65% with age 60 years old.

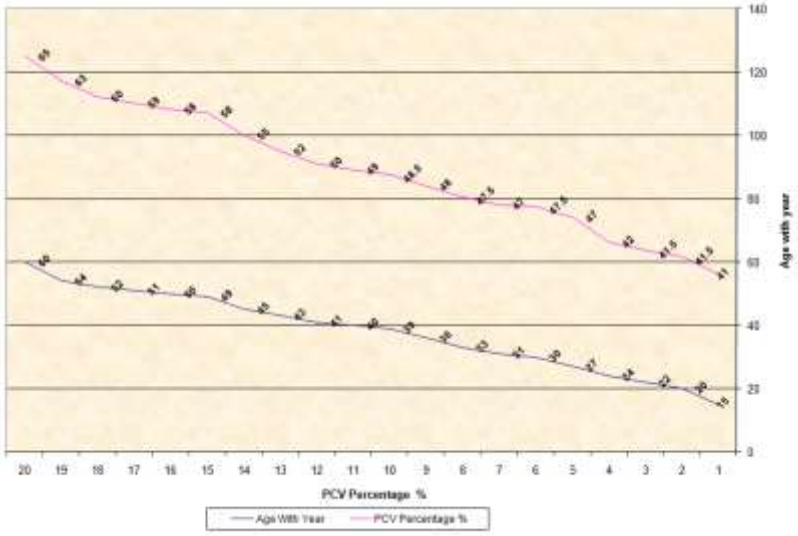

Diagram no 4: PCV percentage for smokers

On the table no (2) below show the result of the relationship between the hemoglobin concentration and the blood viscosity (PCV%) with the age of the non smoker individuals.

Table no (2):- explain the relationship between the hemoglobin concentration and the blood viscosity (PCV%) with the age of the non smoker individuals.

Number of Sample	Age With Year	Hemoglobin concentration Gram / dl	PCV Percentage %
1	15	12 G/ dl	36 %
2	20	12.6 G/ dl	36.5 %
3	22	12.3 G/ dl	36.5 %
4	24	12.6 G/ dl	37 %
5	27	12.9 G/ dl	37 %
6	30	12.7 G/ dl	37.5 %
7	31	13.3 G/ dl	38 %
8	33	13.0 G/ dl	38.5 %
9	36	13.3 G/ dl	38 %
10	39	13.2 G/ dl	39.5 %
11	40	13 G/ dl	39 %
12	41	14.7 G/ dl	40 %
13	43	14.5 G/ dl	42 %
14	45	14.6 G/ dl	42 %
15	49	15.4 G/ dl	42.5 %
16	50	16.3 G/ dl	43 %
17	51	17.3 G/ dl	43.5 %
18	52	16.4 G/ dl	43 %
19	54	17.5 G/ dl	43 %
20	60	17 G/ dl	43.5 %

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On the diagram below the date mention that there are a positive relationship between the hemoglobin and the age of the non smoker individual for that we can see in the diagram no (4) in the 15-20 years old may be the Hb concentration on the normal range, so we can see the Hb concentration directed to increasment when the age become more years old 17-18 g/dL with 60 years old.

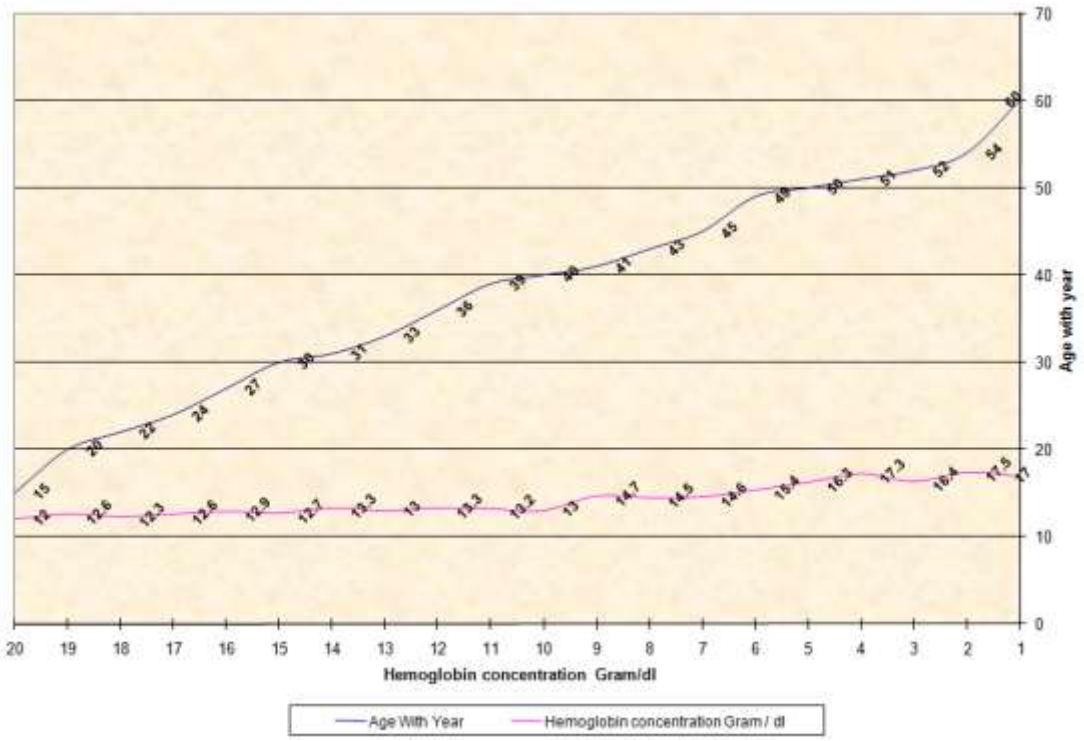

Diagram (4): explain the positive relationship between the hemoglobin and the age of the non smoker individual

Also we can see the positive relations on the diagram No (5) between the PCV% and the age nonsmoker individual for that with the age progress the PCV% stay on the normal range which are 36-50%.

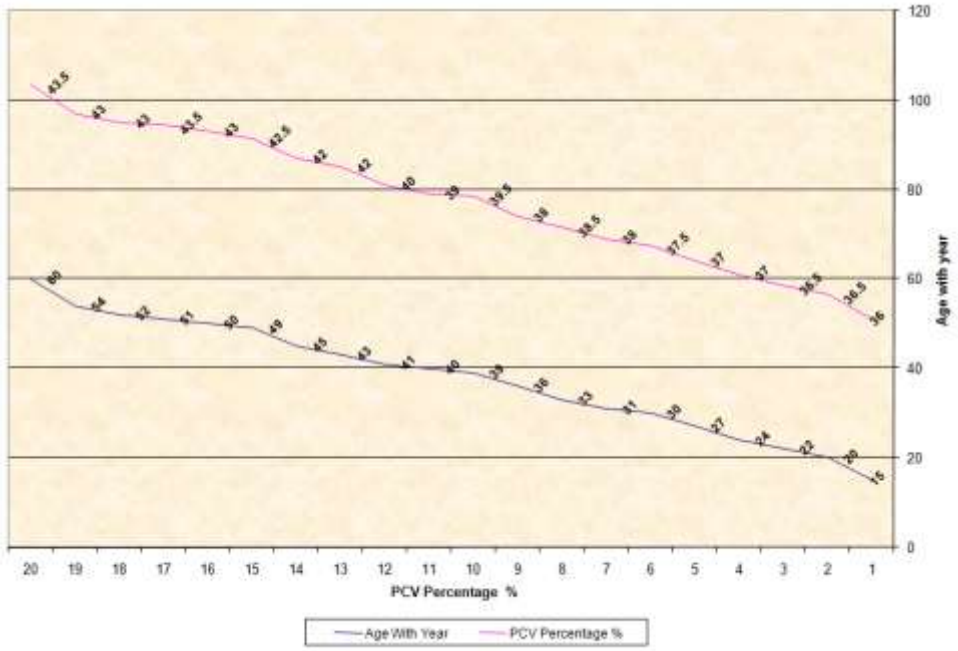

Diagram No (5): explain the positive relations between the PCV% and the age of nonsmoker individual

All to all It is already clear to us that the smoking consider an excellent factor for all types bacterial disease. However, the tobacco of all type of cigarette are a suitable environment to bacterial growth, also the smoking effect on the many indications in the blood like hemoglobin and blood viscosity or PCV% like mentioned above.

Recommendations

First: - At the level of society and the family:

1 - Tip smokers to quit smoking and a statement of his rule legitimate.
2 - convince parents of the need to quit smoking for their children.
3 - Parents must keep their children's behavior.
4 - connect children to mosques and workshops teaching the Koran.

Second: - At the level of the individual:

1- you get rid of smoking and cigarettes.
2- drink a lot of water and juices.
3- exercise and walking a lot.
4- Avoid caffeine in any form.
5- Stay away from Babysitting drinkers smoke.

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