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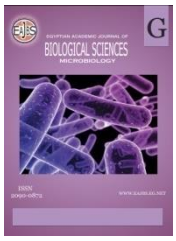

Investigate The Relationship Between *Haemophilus infleunzae* Type and Anemia

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ABSTRACT

The study included measuring the level of Hemoglobin, Haptoglobin, Haemoxin, Transferrin, and Hematocrit (Hct) for eighty six people suffering from upper respiratory tract inflammation whose isolates were diagnosed as *Haemophilus infleunzae* and other bacterial species, in order to compare the concentration of these proteins between Infected with *Haemophilus infleunzae* and other types and its relationship to anemia using ELISA and CBC technique. It was found that patients with *Haemophilus infleunzae* type b had a concentration of hemoglobin, haptoglobin, hemopexin, transferrin, and hematocrit lower than the normal level, and there was a significant difference between them and the people infected with other bacteria whose levels of these proteins and Hct varied, and we show that there is a relationship between infection with *Haemophilus infleunzae* and anemia.

INTRODUCTION

Anemia is a condition in which the number of Red blood cells (Rbc) or the amount of hemoglobin, which is the protein that carries oxygen in them, decreases, anemia results from a lack of iron and vitamins such as vitamin B12, Medicines and some diseases, including hereditary, and infected with bacterial and viral infection and others reasons (Robert and Means, 2019) Some studies indicate that there is a relationship between *Haemophilus infleunzae* and anemia (Williams *et al*., 2009), these studies were built through the need of some types of bacteria such as *Haemophilus infleunzae* for iron, which they use in the process of reproduction and growth in the body of the organism (Braun and Hantke, 2011),the reason is that bacteria do not contain enzymes that contribute to the Heme synthetic pathway, and consequently their inability to produce Protoporphyrin IX (PPIX) (Norskov, 2014), protoporphyrin is an organic compound that contributes to heme formation (Sachar, *et al*., 2016) Thus, bacteria stimulate several receptors on their surface that bind blood proteins to the host, and these proteins are Hemoglobin, Hemoglobin-Haptoglobin, Hemopexin, in addition to secreting Hemophore, which works to gain iron linked to Transferrin through peripheral ion protein binding Periplasmic ferric (Abuga *et al*., 2020), In addition, *H. infleunzae* type b contains the capsid antigen, which is a polyri bosyl ribitol phosphate (PRP) that captures elements from red blood cells (Shurin *et al*., 1986), hence the idea of the research that aims to study the relationship of *Haemophilus* infleunzae bacteria with anemia by measuring the concentration of hemoglobin protein, haptoglobin, hemopexin, transferrin and hematocrit.

MATERIALS AND METHODS

Sampling Collection:

86 blood samples were collected from people with upper respiratory inflammation of both gender and different age groups. 10 ml of venous blood was withdrawn by a single-use medical syringe and the blood was placed in special tubes to collect blood and left the blood for ten minutes at room temperature to coagulate and after This was placed in a centrifuge at 3000 rpm for 10 minutes, then the serum was withdrawn through the micropipette and placed in sterile tubes and kept in a frozen state at -20 ° C until the measurement of the concentrations of hemopexin, haptoglobin, transferrin.

Measurement of the Level of Haptoglobin, Hemopexin, Transferrin Proteins in the Blood Serum:

The collected serum samples were taken and the Haptoglobin, Hemopexin, Transferrin proteins which measure uses the ELISA technique, according to the instructions of the kit through the following steps:

This ELISA kit uses the Sandwich-ELISA principle, where the small ELISA plate available in this kit is pre-coated with a special antibody for the Human Hp, Hpx, TF proteins, the standards or samples are added to the pits of the ELIS, and combined with the specific antibody, and then the peroxidase detection antibody is added Human Hp, Hpx, TF and Avidin-Horseradish Peroxidase (HRP) were respectively added to each pit of the plate and incubated, and the rest of the components were removed by washing and then Substrate solution was added to each pit, then the enzymatic reaction is completed by adding a stop solution, which turns yellow, and then the optical density (OD) is measured spectrophotometrically with a wavelength of 450 nm ± 2 nm, and the OD value is proportional to the concentration of Human TF, and then the concentration of Human TF protein is calculated in samples by comparing the OD of the samples with the standard curve.

Measurement of Hemoglobin and Hematocrit (Hct) in a CBC Blood Analyzer System:

The test was conducted to measure hemoglobin and hematocrit (Hct) by taking blood samples from patients with upper respiratory tract infection and placing it in a tube containing an anticoagulant EDTA, as it works to prevent the blood and other components from clumping together and thus gives a guarantee of blockage of the CBC ducts in In the event that clotted masses of blood components pass and give correct results, then the tube containing blood was inserted onto the CBC device, following the instructions of the manufacturer, and hemoglobin was measured through the presence of a chamber in the device called Hgb flow cell, which is done by adding an analyzer reagent called Azide- free isotonic, which analyzes red blood cells and releases hemoglobin from them, which is measured by a photometer called Sepectrophotometers, Hct was measured and its percentage was calculated mathematically by equations stored in the device, and after waiting for minutes, the results were displayed on the screen in the device and then printed.

RESULTS AND DISCUSSION

Measuring Some Blood Proteins and Estimating the Percentage of Hct in People with Anemia and Its Relationship to Anemia:

The level of some blood proteins Hemoglobin, Haptoglobin**,** Hemopexin and Transferrin was measured, and the Hct test was performed on eighty six people with upper respiratory infection whose isolates were diagnosed as *Hemophilus infleunzae* or belonging to other species.Haptoglobin, hemoglobin and transferrin were measured using ELISA technology, hemoglobin protein measurement, and Hct test using a CBC blood analyzer to make a comparison in testing the ratio or concentration of these blood proteins and Hct between people infected with the *Hemophilus infleunzae* and other types and their relationship to anemia has been divided into five groups:

Group A includes 34 samples of people infected with the genus *Staphylococcus*.
Group B includes 25 samples of people infected with the genus *Streptococcus*.
Group C includes 12 samples of people infected with the bacteria *Klebsiella pneumoniae*.
Group D includes 9 samples of people infected with *Hemophilus infleunzae* type b.
Group E includes 6 samples of people infected with Nontype *Hemophilus infleunzae* bacteria.

Table 1 shows that the percentage of Hct analysis was normal in group A and group B, while it indicated a decrease in group C and group E, and while it indicated a high decrease in a group D as well as Figure 1.

Table 1:- Statistical analysis of Hct. analysis

Group	Hct.Analysis
A	**40.94±1.071a**
B	**40.25±0.851a**
C	**34.50±0.798b**
D	**29.00±1.069d**
E	**33.00±0.894c**

Table 2 shows a normal percentage of hemoglobin protein in group A, followed by group B, then it decreases in group C, and then a further decrease in group E and group D, as shown in Figure 1.

As was showed the table that the routine Haptoglobin ratio was normal in group B and group A, and group C and group E then decreased in a group D, as shown in Figure 1**.**

The table shows that the percentage of Hemopexin protein was normal in group A, group B, and group C, and then it was observed that the protein percentage decreased in group E and group D, as shown in Figure 1. The table showed a normal percentage of Transferrin protein in group B, and group C and decrease in group A, and then a high decrease in group E and group D, as shown in Figure 1.

Table 2:- Statistical analysis of Hb, Hp, Hpx, TF analysis.

Parameter / Groups	Hb	Hp	Hpx	TF
	Mean ± SD	Mean ± SD	Mean ± SD	Me an ± SD
A	14.81±1.880a	127.94±70.592ab	102.64±17.770a	186.06 ± 4.425c
B	14.30±1.559a	153.20±58.020a	95.45±23.659b	263.75 ± 54.423a
C	10.09±0.944b	78.55±35.951bc	87.00±10.507b	231.45 ± 26.504b
D	7.13±1.356c	33.38±39.978c	23.25±8.876d	169.75 ± 3.370c
E	10.50±0.548b	75.50±70c	41.67±1.862c	179.33 ± 3.077c

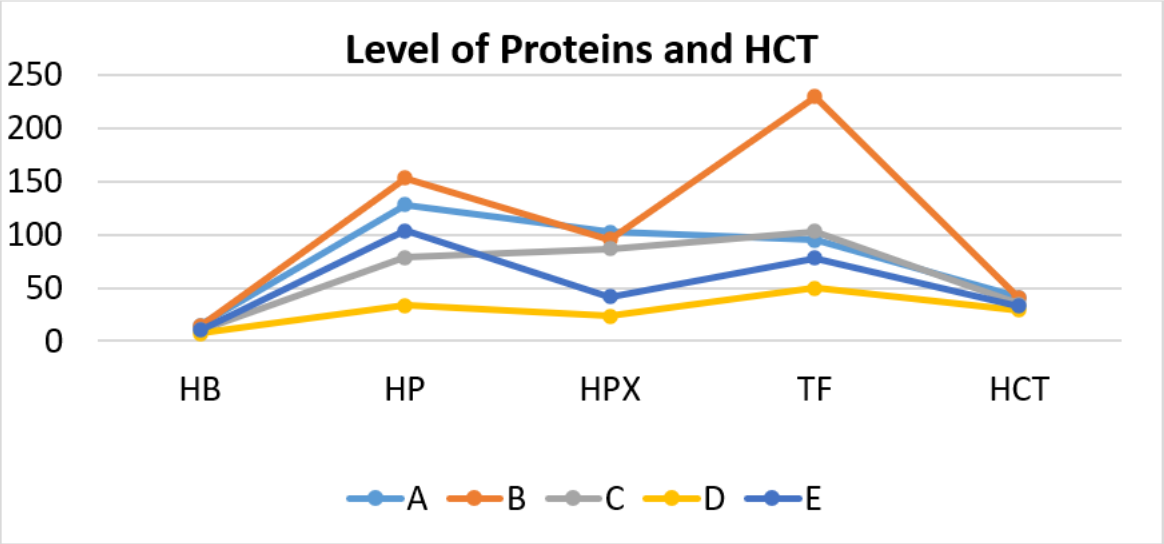

Fig. 1: The level of blood proteins and Hct

Comparison in the Level of Proteins and Hct Between Infected with *Haemophilus influenzae* and Other Species:

It was noted through the results that the percentage of Hct test was highly low in people diagnosed with *Haemophilus influenzae*, especially type b and it was low in those infected with *Klebsiella pneumoniae*, and it was found that the percentage of Hct test was normal in people infected with Staphylococcus and Streptococcus bacteria.

The results showed a significant decrease in the level of hemoglobin protein concentration in people infected with *Haemophilus influenzae*, especially type b, as the bacteria encode several receptors to bind with human blood proteins and acquire hemoglobin from them (Morton *et al*., 2009), for people infected with *Klebsiella pneumoniae*, the percentage of hemoglobin protein was It tends to be below the normal level, meaning it tends to decrease slightly, the bacteria acquire protein due to their possession of Enterobactin, which is one of the manifestations of iron carrier siderophores, which has a high affinity for iron acquisition (Zhu *et al*., 2001). The results showed that the percentage of hemoglobin protein was normal in people infected with *Staphylococcus* and *Streptococcus*.

The level of haptoglobin protein concentration was very low in people infected with Haemophilus influenzae bacteria, especially type b. The researcher Morton *et al* (2006) mentioned that the bacteria *Haemophilus influenzae* type b of strain HI689 contains three genes called hgp, It contributes to the acquisition of haptoglobin, which is a complex of hemoglobin-haptoglobin. The level of haptoglobin in persons infected with *Staphylococcus* and *Streptococcus* and *Klebsiella pneumoniae* tends to normal level and this result agreed with what was obtained by researcher Wasserzug *et al*., (2007) when he was able to measure the percentage of haptoglobin in patients infected with *Streptococcus* bacteria and stated that the percentage of this protein was normal.

As for measuring the level of hemopexin protein, it was found that there was a significant decrease in people infected with *Haemophilus influenzae* bacteria, especially type b, and it is consistent with what Choby *et al*., (2016) mentioned the ability of this type of bacteria to acquire heme from hemopexin, while in people infected with *Klebsiella pneumoniae* and *Staphylococcus* and *Streptococcus*, the protein content was normal

The percentage of transferrin protein in people infected with *Haemophilus influenzae* was significantly low, and these results agree with what the researcher said. Whitby *et al* (2009) and Rodríguez *et al*., (2019) bacteria need this protein, and as it was

observed that this protein was reduced in people infected with *Staphylococcus* bacteria, The researchers Taylor and Heinrichs (2002) mentioned that *Staphylococcus* bacteria have a gene called StbA for protein A that is attached to the wall of the wall. The bacterial cell has the ability to bind to human transferrin, which represents a means by which bacteria are able to access iron. The results showed that the percentage of transferrin protein concentration in people infected with *Streptococcus* and *Klebsiella pneumoniae* tends to be normal, and this result is consistent with what was mentioned by researcher Shooraj *et al*., (2020) that transferrin did not participate in the iron acquisition of these bacteria. It was found from our results that the concentration level of all these Proteins and the Hct test is very low in people infected with *Haemophilus influenzae*, especially type B.

Relationship of *Haemophilus influenzae* Bacteria with Anemia:

It was found that *Haemophilus influenzae* cause anemia through the consumption of hemoglobin, haptoglobin, haemoxin and transferrin proteins (Abuga *et al*., 2020), This is consistent with the results of our current study, which showed a high decrease in these proteins and the Hct test with people infected with *Haemophilus influenzae* bacteria. Infected children, as well as the researcher Shurin *et al*., (1986), found a relationship between *Haemophilus influenzae* type b bacteria and anemia by measuring the level of hemoglobin and haptoglobin protein for about 43 individuals infected with these bacteria at different ages ranging from 6 to 20 years and showed about 27 An individual with anemia after finding a decrease in the level of these proteins and after years, the relationship between *Haemophilus influenzae* bacteria and anemia was also proven when researchers William *et al*., (2009) and Kato *et al*., (2017) isolated this bacteria from children suffering from anemia in African countries, including Kenya, and their ages ranged between 1- 14 years old and had a low hemoglobin level. From the above results and what we have reached, one of the important. That these bacteria cause anemia may be due to their great need for iron to grow aerobically, in addition to heme or protoporphyrin IX, but they are unable to manufacture it because they lack enzymes that are found in the biosynthesis pathway of the porphyrin ring (PPiX). Iron in PPiX by having a gene that encodes the ferrochelatase enzyme to form heme. However, it is not believed that there is an important extracellular source for the free PPiX pathway that occurs in the body of the organism, because the ability to grow aerobic is important for Haemophilus influenzae to be an invasive pathogen, and therefore the bacteria must gain heme from sources The host to maintain the invasive infection, and it possesses receptors in the cell membrane that stimulate it to bind to blood proteins (hemoglobin, haptoglobin, hemopexin, transferrin) through its association with free hemoglobin that binds to haptoglobin and extracellular hemopexin derived from lysed blood cells (Seale *et al.*, 2006), and the bacteria's need for iron was proven to be significant by not growing in culture media except with the presence of factor X (Hemin), which It is essential in the process of iron synthesis and is present in respiratory enzymes such as Catalase, Peroxidase, Cytochrome oxidase, Factor V ((Nichotineamide Adenine Dinucleotide) and is an enzymatic co-enzyme required for its presence in oxidation-reduction reactions (Connie and Henderson 2011; Almelan, 2015)

Conclusions

Our current study showed the relationship between *Haemophilus influenzae* type b and anemia by measuring the blood proteins hemoglobin, haptoglobin, haemoxin and transferrin, and the Hct test and it was found that this type of bacteria has a role in causing anemia During his acquisition of these proteins, which showed test results at low levels, and in addition to the low percentage of Hct test, it reflects the rest of the other bacterial species that showed test results at varying levels.

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