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Comparative study on some haematological parameters on faecal occult blood subjects and apparently healthy subjects

Okoroiwu, Ijeoma Leticia[1*], Obeagu, Emmanuel Ifeanyi[2], Ochei, Kingsley Chinedum[3], Christian, Serekara Gideon[4] and Elemchukwu, Queen[4]

[1]Departement of Medical Laboratory Science,Faculty of Health Sciences,Imo State University, Owerri, Nigeria
[2]Diagnostic Laboratory Unit,Michael Okpara University of Agriculture,Umudike,Abia State, Nigeria
[3]Department of Medical laboratory Science,Ambrose Alli University,Ekpoma,Edo State, Nigeria
[4]Rivers State College of Health Science and Technology, Rumueme, Port Harcourt, Nigeria
Corresponding author

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A B S T R A C T

A comparative study on some haematological parameters of subjects presented with faecal occult blood and those of apparently healthy subjects was carried out in Owerri, Imo state Capital. A total of 50 subjects who were positive for faecal occult blood and 25 faecal occult blood negative subjects were studied. The aim was to assess the effect of faecal occult blood may have on the parameters. The faecal occult blood was determined using immunological method while haematological parameters were determined using standard haematological techniques. The mean results obtained were as follows: Total WBC for 3.34±0.70, control 7.09±3.36. Statistical analysis of the two results shows significant difference ($p<0.05$). Packed cell volume for FOB subjects 22.54±3.66, control 37.24±4.91 significant statistically difference was equally observed between the two ($p<0.05$). Result obtained from platelet count was 120.8±23.90 and 248.96±71.53 respectively for FOB and control subjects. Significant statistical difference was observed between the two values ($p<0.05$). Conclusively, faecal WBC, PCV and platelet count negatively.

Introduction

The word "Occult" means hidden from view. The occult blood test is performed as part of the routine physical examination during the examination of the rectum. It is used to detect microscopic blood in the stool and is a screening test for colorectal cancer. The fecal occult blood (FOB) refers to blood in the feces that is not visibly apparently. A

fecal blood test (FOBT) checks for hidden occult blood in the stool (feces). Newer tests look for globins, DNA or other blood factors including transferrin, while conventional stool guaiac test look for heme (Lippincott and Wilkins, 2006).

Occult bleeding has many causes as other forms of more rapid gastrointestinal bleeding such as rectal bleeding (passage of red blood or blood clots rectally) and melaena (black tarry stool as a result of bleeding from upper intestines. The fecal occult blood test can detect bleeding from almost anywhere along the length of the digestive tract resulting from several conditions such as peptic ulcer, stomach cancer, esophagitis, ulcerative colitis, colorectal cancer or polps and hemorrhoids (Maton Lawrence *et al.,* 2006).

The result also can be positive when someone has been taken aspirin or other medications that irritate the digestive tract (Ochei and Kolhatkar, 2007). Primarily, occult blood test is also done to detect or prevent colon cancer in people without intestinal symptoms. Cancers of the colon are common and frequently produce fecal occult blood long before they cause other symptoms such as abdominal pains, rectal bleeding or changes in bowel habits. Cancer of the colon is the disease characterized by the development of malignant cells in the lining or epithelium of the first and longest portion of the large intestine. Malignant cells lost normal control mechanism governing growth. These cells may invade surrounding local tissue or they may spread throughout the body and invade other organs system. Synonyms for the colon include the large bowel of the large intestine. The rectum is the continuation of the large intestine into the pelvis that terminates in the anus (Beckett *et al.,* 1996).

Certain food and medication can influence the test results. Some fruits contain chemical that can affect the test paper from reacting with the blood. Aspirin and some NSAIDS irritate the stomach resulting in bleeding. Many vegetables and red meat, fruits containing vitamin C also should be avoided for a specified period of time before the test as all of these factors could result in a false-positive result (Green *et al.,* 2002).

The main of this study includes, to determine the values of platelets in occult blood positive subjects. And to determine the level of packed cell volume and white blood cells in occult blood positive subjects. Then, compare the variables determined with those of apparently healthy subjects.

Materials and Methods

Study area

The study was conducted in Owerri, the capital city of Imo State. Owerri metropolis is located in the tropical rain forest of south-east of Nigeria, lying on latitude 5˚41□-5˚42□N and longitude 7˚13□-7˚15□E. It has an estimated population of about 350,000. Christianity is a dominant religion in Owerri, although, few Muslims and pagans are sighted within the area. The climate is tropical with a mean daily temperature of 29% for most of the year. The annual rainfall is between 217 and 240cm with destruct wet and dry.

Ethical clearance

Consent was sought from the relevant authorities, the head, department of medical Laboratory Science, Imo State University Owerri. In the area of sample collection from the subjects, informed consent was sought. Adequate verbal information was provided for the subjects, letting them know

the need and essence of collecting their blood and stool samples and the nature of the research work.

Selection of cases

The number of cases is based on the following criteria.

1. Abnormal pain.
2. Rectal bleeding.
3. Changes in bowel habit.
4. Loss of appetite.
5. Black Stool.
6. Weight Loss.
7. Retching after eating.
8. Indigestion like pain.

Sample size

Sample size used for this survey was seventy five. These were grouped into:

Group 1: Comprised fifty subjects who responded positively for fecal blood occult test.

Group2: comprised twenty five apparently healthy subjects (control).

Sample collection and method

With testing kits, a small sample of stool is collected making sure it is not contaminated with urine and water. The stool is placed on a dry container properly labeled with a name, date and time of collection.

Method

The methods used for the test were gotten from Ochei and Kolhatker (2002). All reagents were commercially purchased and their standard operating procedure was adhered to.

Immunological testing (Chika and Masayoshi, 2004)

Principle:

A sample of stool is mixed with a solution that contains antibody to globin, 30 the protein part of the hemoglobin molecule. The antibody is combined with a small amount of gold. When the antibody / gold complex bind to the globin in the stool, the antibody / gold /globin complex settles out of the solution as a visible line on the test stripe.

Procedure

1. A small sample of the stool was emulsified on the test area of the stripe of the fecal blood occult test stripe.

2. A drop of solution was added to it and allows reaction to take place.

3. Double line signifies positive, while a single line signifies negative.

Packed cell volume

Method: Microhaematocrit method (Dacie and Lewis, 2007).

Principle: the packed cell volume is the production of whole blood occupied by red cells, expressed as a ratio (1/1). Anticoagulant blood in a glass capillary of specified length, bore size, and well-thickness, is centrifuged in a microhaematocrit centrifuge at 12000–15000 rpm for 3–5 minutes to obtain constant pacing of the red cells. A small amount of plasma remains trapped between the packed red cells. The PCV value is read from a scale haematocrit reader calculated by dividing the height of the red cells

column by the height of the total column of blood.

Procedure

1. A capillary tube was about three quarter filled with well mixed EDTA anticoagulant.

2. The other unfilled end was sealed with sealant plasticine.

3. It was carefully placed in one number slots of the microhaematocrit rotor with the sealed end against the rim gasket and centrifuge for 5minutes at 12000rpm.

4. The result pf PCV were read using the microhaematocrit reader.

Total White Blood Cell

Method: Neubauer Counting Chamber (Cheesbrough, 2000)

Principle: Whole blood is diluted 1 in 20 in an acid reagent which haemolyzes the red cells (not the nucleus of the nucleated red cells), leaving the white cells to be counted. White cells are counted microscopically using an improved neubauer ruled counting chamber and the number of WBCs per Liter of blood calculated.

Procedure

1. 0.38ml of diluting fluid was dispensed into a small container or tube.

2. 20µ/l (0.02ml) of well mixed EDTA anticoagulated venous blood was added into the container containing the diluted fluid.
3. The counting chamber was assembled.
4 The diluted blood sample was re-mixed using a Pasteur pipette held at an angel of about 45 degree, one of the grids of the chamber was filled with the sample.

5. The chamber was left undisturbed for 2 minutes to allow time for the white cells to settle

6. The chamber was placed in a Petri-dish on damped tissue or cotton wool to prevent drying of the fluid.

7. The underside of the chamber was dried and placed on the microscope stage using 10x objectives.

8. The cells in the four large squares of the chamber marked w1, w2, w3, w4 was counted.

9. The number of white cells per liter of blood was reported.

Platelet count

Method: Neubauer Counting Chamber (Cheesbrough, 2000)

Principle: The blood was diluted 1 in 20 in a solution of ammonium oxalate reagent which lysed red and white blood cells. Platelet was counted microscopically using an improved neubauer counting chamber and the number of platelet per blood was calculated.

Procedure

0.38ml of 1% ammonium oxalate diluting fluid was dispensed into a small test tube and 0.02ml of blood sample (anticoagulated) was added and the contents mixed evenly. The chamber was well charged and placed in a Petri-dish containing blotting paper and

covered with a lid to avoid drying. The underside chamber was placed on a microscope stage, using 10x objective, the ruling of the chamber was focused and centre square was brought into view. 40x objective was used to focus small sized platelets which were seen as small bright fragment. The platelets was counted in the small square and the number in 1 litre of blood was reported and multiplied by ten (10) to give the actual number of platelets counted.

Results and Discussion

Table 1 shows the mean concentration values of total white blood cell in healthy individuals and fecal occult blood patients. From the results obtained, there is significant difference ($p < 0.05$) in total white blood cell of fecal occult blood patients when compared with healthy individuals (control).

Table 2 above shows the mean concentration values of packed cell volume in the fecal occult blood subjects and healthy individuals (control). From the results obtained, there is a significant difference ($P<0.05$) between the patients when compared with the healthy individuals (control). Table 3 shows the mean concentration values of platelet count in fecal occult blood subject and healthy individuals. From the results obtained, there is a significant difference ($p < 0.05$) between the platelet counts of fecal occult blood patients when compared with the healthy individuals (control).

A comparative study of total white cell count, packed cell volume and platelet count was carried out on subjects positive for faecal occult blood and those negative for fecal occult blood. The aim was to assess the effect of FOB on the parameters. The result obtained for total white cell count on FOB subjects and non FOB subjects were 3.34±0.70 and 7.09±3.36 respectively. The results show significant statistical difference $P<0.05$. This study recorded reduction in total white cell count in some past studies. Leucocytosis were reported. The differences may have resulted from the extent of bleeding, duration, causes and management. Some inflammatory drugs could cause neutropenia as observed in this study. The FOB may have been associated with infection by bacteria, viral, protozoal or such types that can cause relative leucocytosis. The leucopenia observed may have also be caused by bone marrow suppression.

The packed cell volume for FOB and non Fob subjects were 22.54±3.66 and 37.24±4.91 respectively. There is significant statistical difference between the two values ($p<0.05$). The reduction observed in PCV in this study could be due to anaemia which may have been caused by iron depletion usually caused by chronic bleeding. This depletion may have caused ineffective erythopoiesis in the FOB subjects. This is because the best common cause of iron deficiency is blood loss which may be physiological or pathological. Chronic disease may have been the cause of low PCV observed on this study as this may be the possible cause of the FOB in subjects.

The platelet count values obtained for FOB and non FOB subjects were 120.8±23.90 and 248.96±71.53 respectively. There is again significant statistical difference between the two values. Abnormal haemostasis results in some sorts of bleeding and platelet deficiency is the most common causes of bleeding. The leucocytosis may have resulted from marrow oplasia caused by drug, virus etc.

This also depends on the cause of the bleeding. Platelets are used up as it functions in adhesion, aggregation and formation of

platelet plug in its effort to arrest the bleeding.

The conclusion of this study is FOB results in anaemia causing reduction in packed cell volume. It also affects the white cell count as well as the platelet count. These parameters could be used in diagnosis prognosis and management of FOB.

Table.1 Comparison of the mean concentration of the total white blood cell in the fecal occult blood subjects and healthy individuals (control)

Parameter	FOB (subjects) n=50	Non FOB (control) n= 25	p-value
Total WBC ($X10^9/L$)	3.34 ± 0.70	7.09 ± 3.36	P <0.05

All values were recorded as mean ± standard deviation (x ± SD)

Table.2 Comparison of the mean concentration of the packed cell volume in the fecal occult blood subjects and healthy individuals (control)

Parameter (%)	FOB (subject) n=50	Non FOB (control) n= 25	P-value
Packed cell volume (%)	22.54 ± 3.66	37.24 ± 4.91	P < 0.05

All values were recorded as mean ± standard deviation (x ± SD).

Table.3 Comparison of the mean concentration of platelet count in fecal occult blood patients and healthy individuals (control)

Parameter	FOB (subjects) n = 50	Non FOB (control) n = 25	P < value
Platelet ($x10^9/L$)	120.8 ± 23.90	248.96 ± 71.53	P < 0.05)

All values were recorded as mean ± standard deviation (x ± SD)

Recommendations

It is recommended that more work be done on this topic, assessing other haematological parameters. Differential white cell count should be done to ascertain which of the cells is mostly affected.

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