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Some hematological changes in males suffering from polycythemia vera in Babylon province

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Abstract

The present study was designed to investigate changes occurring in some hematological parameters of males suffering from polycythemia vera. The total number of patient males was 50, while the number of control males was 20. All ages of subjects ranged between 45 years to 65 years.

It was found that the levels of packed cell volume (PVC), hemoglobin concentration (Hb), red blood cells count (RBCs), total white blood cells count (WBCs), blood platelets, and reticulocytes pointed out a significant increase ($P<0.01$) in males affected with polycythemia when compared with control males, whereas, the levels of erythrocytes sedimentation rate pointed out a significant decrease ($P<0.01$) when compared with control. The changes summarized above may attributed to excess production of red blood cells entering blood circulation.

الخلاصة

صممت هذه الدراسة لفحص بعض التغيرات الحاصلة في بعض المعايير الدموية في الذكور المصابين بفرط كريات الدم الحمراء. تضمنت هذه الدراسة فحص 70 من الذكور، 50 منهم مصابين بفرط كريات الدم الحمراء و 20 اصحاء.

بينت نتائج هذه الدراسة ارتفاعاً معنوياً بمستوى ($P<0.01$) في قيم كل من حجم الخلايا المضغوط PCV وتركيز خضاب الدم Hb والعدد لكريات الدم الحمراء RBCs، والعدد الكلي لكريات الدم البيض WBCs والصفيحات الدموية blood platelets والخلايا الشبكية reticulocytes في الذكور المصابين عند مقارنة النتائج مع الذكور الاصحاء.

لوحظ بأن قيم معدل سرعة ترسيب كريات الدم الحمراء ESR قد حققت انخفاضاً معنوياً بمستوى ($P<0.01$) في الذكور المصابين عند مقارنتها بالذكور الاصحاء ان التغيرات الحاصلة اعلاه يمكن اعزاؤها بصورة رئيسة الى الانتاج العالي لكريات الدم الحمراء بصورة غير مسيطر عليها فسيولوجياً.

Introduction

Historically, the clinical phenotype of polycythemia vera (PV) (Plethora, engorged vein) was appreciated long before the disease was formally described by Vagues, 1982 and subsequently by Osler in 1903 (Tefferi, 2003).

Dameshek, 1941 was classified PV as a chronic myelo proliferative disorder (CMPD) along with other related myeloid disorders, including chronic myeloid leukemia (CML) and angogenic myeloid metaplasia (AMM). This disease was found to be the most common among Jews of European extraction than among most non-Jewish population (Marchioli *et al.*, 2005).

Generally, PV is a disorder of multipotent hematopietic stem cell that manifests as excess production of normal erythrocytes (Hoffbrand *et al.*, 1999). It is grouped with Philadelphia chromosome negative myeloproliferative disorders and can be differentiated from then by the predominance of erythocytes production (Hoffman *et al.*, 2007).

Erythrocytosis, on the other hand, tell us that the RBC count in blood is higher. It need not associated with increased hemoglobin content (Krishna, 2004).

PV must be differentiated from other causes of polycythemia. The polycythemias can be divided by etiology into three groups as follows:-

1- Apparent or relative polycythemia is due to a decrease in plasma volume with normal red cell mass. This type of polycythemia is associated with dehydration, obesity, and stress (Pearson, 1999).

2- Primary polycythemia or absolute polycythemia, which refers to an actual increase of red cells and their hemoglobin content. This type is caused by intrinsic hyperproliferation of hematopoetic stem cell independent of erythropoietin (EPO) stimulation or with an exaggerated response to low EPO level. The most common primary polycythemia is PV in which the hematopoietic stem cell proliferates independently of EPO or EPO receptors become equisietly sensitive to the normal level of EPO (Prchal and Axelard, 1974).
3- Secondary polycythemia, is due to elevated levels of EPO that induce erythrocyte proliferation. At the time of presentation, the increased RBC mass may have reached an equilibrium, and the EPO level is often within normal limits. However, the patient's normal EPO level is inappropriately high for the elevated hematocrit. High EPO concentrations are due to physiologically appropriate causes (Vgo *et al.*, 2004).

Hypoxemia is physiologically appropriate secondary polycythemias result from hypoxia. Hypoxia is the common endpoint of various causes of physiologically appropriate secondary polycythemias. Exposure to exogenous EPO is physiologically inappropriate polycythemia is often due to exogenous sources of EPO such as those seen in malignancies and endocrine disorder (Najean *et al.*, 1998).

Materials and Methods

Materials

The subjects of the study

This study was applied over a 5 months period from October 2007 to March 2008. A total number used was 70, patients and healthy subjects of males. The total number of male patients was 50, while the number of healthy males was 20. The ages of all subjects ranged between 45 years to 65 years. All patients were attending the bank of blood in Babylon for treatment and restored the packed cell volume to normal range through reduction of excess blood. All patients of this study were non smoking and free from other chronic disease.

Methods

The collection of blood was carried out in bank of blood in Babylon. Before collection, the patients were asked to rest on a chair for approximately half hour. Collection was always performed between 9-11 a.m. by using venipucture needles.

A-Hematological Parameters

1- Red blood cells count (RBCs count):-

Blood was diluted with formal citrate solution (1% formalin in 38 g/L trisodium citrate). 20 microliter of blood was added into four ml of diluting fluid, after mixing by a mechanical mixture; the neubaur hemocytometer chamber was filled before being examined under the microscope to RBCs count (Dacie and Lewis, 1995).

2- Total white blood cells count (WBCs count):-

Blood was diluted with 0.4 ml of Turk's solution (1m of glacial acetic acid, 2 ml of gentian violet, and 100ml of distilled water). A twenty microliter of blood was added and mixed in a mechanical mixture; the counting a Neubaur Chamber was used to count the total WBCs (Dacie and Lewis, 1995).

3- Determination of packed cell volume (PCV):

Microhematocrit method was used. A heparanized capillary tubes were used. Tubes were permitted to fill to approximately three quarters of its length and then the unmarked end is closed with modeling clay and put in microhematocrit centrifuge (Dacie and Lewis, 1995).

4- Estimation of hemoglobin (Hb):-

The Hb was estimated by using the cyanmethaemoglobin method. The method was based on Drabkin's cyanide-ferricyanide solution (Markarem, 1974).

5- Erythrocytes sedimentation rate (ESR):-

Westergren method was used to determine the ESR. Blood was diluted with trisodium citrate solution 3.8%. 0.5ml of diluted solution was used for 2ml of blood, and the mixture was mixed, and then drown into Westergren tube up to the zero mark and tub set upright in a stand position. The level of the red blood cells column is read at the end of one hour (Dacie and Lewis, 1995).

6- Determination of the blood platelets count:-

Blood was diluted with ammonium oxalate solution (10gm of ammonium oxalate and 1000ml of distilled water). 20 micro liter of blood was added into 4 ml of dilution fluid. The diluted mixture was mixed and a Neubour hemocytometer chamber was filled and left for 15 minutes in a petridish chamber containing a moistened filter paper. The sample was examined under 100x objective lens of microscope to count blood platelet. (Pittiglio and Sacher, 1987).

7- Determination of reticulocytes:-

Blood was diluted with new methylene blue staining solution (0.5 gm of new methylene blue, 1.6 gram of potassium oxalate, and 100 ml of distilled water). Equal amounts of blood and staining solution were added in test tube and then mixed. The mixture was drawn into capillary tube and remained for 10 minutes at a room temperature. A thin smear was prepared from blood-dye mixture by application one small drop. The smear was examined under oil immersion through count 1000 red cell in consecutive immersion field and the number of reticulocytes was recorded (Pittiglio and Sacher, 1987).

B-Statistical Analysis

All values were expressed as means ISE. Student's t-test was used to examine the differences between different groups.

Results

Results of packed cell volume (PCV), hemoglobin concentration (Hb), red blood cells, count (RBCs), total white blood cells count (WBCs), blood platelets, reticulocytes, and erythrocytes sedimentation rate (ESR) in males affected with polycythemian were significantly different at a level ($P<0.01$) when compared with healthy control. (Table 1)

Results of PCV of patient males and healthy males were 0.50±0.004, 0.420±0.004 respectively.

Results of Hb of patient males and control males were 17.727±0.332, 12.909±0.284 mg/dL respectively.

Values of RBCs of patient males and control males were 6.745±0.217, 4.718±0.125 cell/ mm^3 respectively.

Results of WBCs of patient males and control males were 8.627±0.381, 4.790± 0.207 cell/mm^3.

Results of blood platelets of patient males and control males were 407.272±19.962, 242.727±20.14 cell/mm^3 respectively.

Results of reticulocytes of patients males and control males were 3.363±0.337, 1.541±0.283% respectively. Results of ESR of patient males and control males were 2.00±0.406, 4.909±0.653 mm/h respectively.

Table (1): shows the mean of packed cell volume (PCV), hemoglobin concentration (Hb), red blood cells count (RBCs), total white blood cells count (WBCs), blood platelets, reticulocytes, and erythrocytes sedimentation rate (ESR) in males affect with polycythemia vera.

Parameter	Patient males	Control males
PCV %	0.5090±0.004	0.420±0.004
Hb gm/dL	17.727±0.332	12.909±0.284
RBCs million/mm^3	6.745±0.217	4.718±0.125
WBCs cell/mm^3	8.627±0.381	4.790±0.207
Blood platelets cell/mm^3	407.272±19.962	242.727±20.143
Reticulocytes%	3.363±0.337	1.541±0.283
ESR mm/h	2.00±0.406	4.909±0.653

-Values are means ± standard error.

- Means are significantly different at $p<0.01$.

Discussion

Results obtained from this study explain increase ($P<0.01$) of red blood cells count (RBCs), total white blood cells count (WBCs), and platelets count.

It should be noted that, in polycythemia vera a single clonal population of erythrocytes, granulocytes, B-cell, and platelet arises when hematopoietic stem cells gain a proliferative advantage over other cells in bone marrow. Also, it has been found, that the erythroid colonies formed from the hematopoietic stem cell in setting of polycythemia vera to grow without erythropoietin (EPO) (Fisher *et al.*, 1994).

Although the colonies do not require EPO, they remain responsive to it. Infact, the EPO receptor is normal, without defects in their functions and quantity (Adamson *et al.*, 1976).

By using Genome-wide scaning, previous studies, showed that in clonal polycythemia vera cells and non-clonal cells formed from the same individual a loss of hetrozygosity in chromosome 9P such a loss was found in approximately 30% of patients with polycythemia vera. This is not classic chromosomal deletion, but rather a duplication of portion of chromosome and the loss of corresponding parental region (Hoffman *et al.*, 2007).

The process is called uniparental disomy. The 9P arm contains a gene encoding for the JAK2 tyrosine kinase. JAK family of kniases is critical in cytokine receptor signaling and transmits the activating signal in EPO-EPO receptor signaling pathway, and inhibition of JAK2 eliminates the EPO independence of erythroid progenitors (Kralovics *et al.*, 2005).

Following these observations was the identification of a loss-of-function somatic mutation in an autoinhibitory JAK2 domain. The mutation essentially produces a gain-of-function affecting the kinase and this occurs when point mutation lead to a valin-to-phenylalanine mutation at colon 617 of JAK2 gene (Thurmes and Steensma, 2006).

The JAK2 V617 mutation leads to constitutive phosphorylation activity and the recruitment of single transducer and activator of transcription molecules provide the necessary proliferative advantage seen in polycythemia vera. This process occurs in the absence of EPO and accounts for both the EPO-independence and EPO-hypersensitivity of polycythemia vera colonies (Bxter *et al.*, 2005).

Values of reticulocytes recorded a significant elevation ($P<0.01$) in males affected with polycythemia vera in a comparison with healthy control. It should be noted that erythropoiesis occurs in bone marrow over a period of about four to five dayes through successive morphologic alterations in nucleated cells from rubriblast to metarubricyte, followed by a non-nucleated polychromatophilic erythrocyte (reticulocyte) that is released into blood circulation to mature into hemoglobin containing erythrocyte in on to two days (Pittiglio and Sacher, 1987).

As explained previously, a clonal population of erythrocytes gain a proliferative fashion over other normal cells in bone marrow, and, at the same time, increase sensitive to normal levels of EPO lead to enhancement of erythropoiesis and more release of immature red blood cells (reticulocytcs) into circulation (Adamson *et al.*, 1976).

Results of packed cell volume (PCV), hemoglobin concentration (Hb), and erythrocytes sedimentation rate (ESR) pointed out a significant increase ($P<0.01$) in males affected with polycythemia vera.

As we know, that the three above parameters (PCV, Hb, ESR) are affected with the production activity and number of RBCs. So, on the basis of physiologyical view, elevation of number of RBCs is accompanied with increase of PCV and Hb associated with decrease values of ESR (Dacie and Lewis, 1997).

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