

## Hematological, Immunological and Biochemical Evaluation in Patients with Secondary Polycythemia.

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(Received: 6 / 3 / 2011 ---- Accepted: 27 / 9 / 2011)

### Abstract

The aim of this study was to investigate whether the relationships between Hematological, Immunological, Biochemical parameters and heavy cigarette smoking in patients with secondary polycythemia, to reach such aim, the study included 60 individuals. They were distributed on two main groups. Group one included 20 healthy non inhaling and non smoking males. Many results were significantly different between smoker patients and healthy non smokers. The levels of hemoglobin, mean corpuscular hemoglobin, total count of red blood cells, leukocytes, platelets and total cholesterol, triglyceride were significantly higher, also the percentage of positive cases for C-reactive protein (CRP) was significantly increased and reached (100%), the level of high-density lipoprotein cholesterol (HDL-C) and immunoglobulin levels of IgG, IgA and complement component C<sub>3</sub>, C<sub>4</sub> were significantly lower in smoker patients than in male non smokers.

**Key words:** Secondary Polycythemia, heavy smoking, hematological parameters, immunological parameters, biochemical parameters.

### Introduction

Polycythemia or erythrocythemia is a condition in which there is ante increase in the total number of blood cells, primarily red blood cells, in the body. The over production of red blood cells may be due to a primary process in the bone marrow (a so-called myeloproliferative syndrome) or it may be a reaction to chronically low oxygen levels and another condition(1). There are two types of primary and secondary polycythemia. The first type occurs when an excess red blood cells are produced as result of an abnormality of the bone marrow(2). While secondary polycythemia is caused by either natural or artificial increases in the production of erythropoietin, hence an increased production of erythrocytes and in turn to increased red cell mass (3). Secondary polycythemia in which the production of erythropoietin increases appropriately is called physiologic polycythemia. This physiologic (meaning normal) polycythemia is a normal adaptation to living at high altitudes. Other causes of secondary polycythemia include smoking, renal or liver tumors, hemangioblastomas in the central nervous system, heart or lung diseases that result in hypoxia, and endocrine abnormalities including pheochromocytoma and adrenal adenoma with Cushing's syndrome. Peoples whose testosterone levels are high because of the use anabolic steroids, including athletes who abuse steroids and people whose doctors put them on doses that are too high, as well as people who take erythropoietin may develop secondary polycythemia (4). The face of these patients appears dusky-red or pigmented, the pulse is full, furthermore, those persons suffer from hypertension, joint stiffness and swelling. Laboratory studies have revealed an increased Hb level (>16g/dL) and hematocrit (>55 ml/ 100 ml)(3). The present study undertaken to investigate whether the relationship between some Hematological, Immunological, biochemical parameters and heavy cigarette smoking in patients with secondary polycythemia.

### Materials and method

Forty males patients with secondary polycythemia aged between (30-58) years who participated in annual health examination performed in Tikrit teaching hospital in 2009. Before the health examination the subject filled out self administered questionnaires about medical history and lifestyle including daily consumption and period of cigarette smoking ;(those who smoking  $\geq$  20 cigarettes per day for ranged 6-35 years. Twenty normal healthy non inhaling and non smoking males were subjected to the study as a control group. Venous blood samples from patients and controls were withdrawn. A sufficient amount could be collected in an anticoagulant container for blood test. Others separated from blood cell using centrifugation technique and used for immunological and biochemical determinations. Estimation of blood tests that included red blood cells (RBCs), hemoglobin(Hb), Mean corpuscular hemoglobin(MCH), total and differential account of WBC and platelets (PC) were performed by using cell tac  $\alpha$  detectors. The concentration of immunoglobulin's IgA, IgG, IgM and complements C<sub>3</sub>, C<sub>4</sub> were estimated in the sera of patients and control using a single radial immunodiffusion assay (RID) by immuno -kits (Biomaghreb, Tunissia)(5). Qualitative serum C-reactive protein (CRP) was determined using (CRP-LINEAR/SPAIN) kits (6) as recommended by the manufacturer. Serum concentration of cholesterol (TC) and high density lipoprotein -cholesterol (HDL-C) were determined by using (Randox/UK) kits (7,8). Statistical analysis was Performed using Spss. Analysis of quantitative data was done using student 's t test and ANOVA. While for qualitative data used Chi-Square test. The results were expressed as means  $\pm$  SD (standard deviation of the mean). P values less than 0.05 was considered to be significant.

**Result**

Table (1) shows comparison of numerical variables in haematological parameters between smoker's polycythemia and control (there was significant increase  $P<0.01$ ) in concentration of Hb in smoker's polycythemia and it reached  $(16.58 \pm 1.05 \text{ gm/dl})$ . RBCs, PC count and MCH showed also significant increase ( $P<0.05$ ) in smoker's polycythemia ( $5.90 \pm$

$0.42, 323.368 \pm 130.96 \times 10^3/\text{mm}^3$ ,  $28.5 \pm 2.24$  Pg respectively).

There found significant increase in total WBCs count ( $10525 \pm 2066.37$  cells/cu. mm. blood) and in differential WBCs that included neutrophils and monocytes ( $6978 \pm 1626.06$ ,  $P<0.01$  and  $701.55 \pm 105.78$  cells/cu. mm. blood,  $P<0.05$ ), while lymphocytes count showed no significant increase.

**Table 1 : Comparisons in hematological parameters between smoker's polycythemia and control (non smoking )**

HAEMATOLOGICAL PARAMETERS	SMOKER'S POLYCYTHEMIA	CONTROL(NON SMOKING)
Hemoglobin (gm/dl)	$16.58 \pm 1.05^{**}$	$13.64 \pm 0.71$
Red blood cells count( cells / cu . mm .blood	$5.90 \pm 0.42^*$	$5.21 \pm 0.39$
Platelets count ( $\times 10^3/\text{mm}^3$ )	$323.368 \pm 130.96^*$	234.363
Mean corpuscular hemoglobin (Pg)	$28.5 \pm 2.24^*$	$26.34 \pm 1.0$
Total white blood cells count (cells .cu. mm .blood )	$10525 \pm 2066.57^*$	$8160 \pm 934$
Neutrophils count (cell. Cu. mm. blood )	$6978 \pm 1626.06^{**}$	$4934.98 \pm 499.23$
Monocytes count (cells. Cu. mm. blood )	$701.55 \pm 105.78^*$	$529.58 \pm 242.05$
Lymphocytes count (cell. Cu. mm. blood )	$2820.788 \pm 996.21$	$2245.02 \pm 788.57$

Mean  $\pm$ SD (standard deviation) ; \* $P>0.05$  , \*\* $P<0.01$ .

The total concentrations of immunoglobulin and complement ( $C_3, C_4$ ) in smoker's polycythemia and control were listed in table 2. It was clear that smoker's polycythemia had significantly lower IgG antibodies compared to control ( $864.75 \pm 159.51$  vs.  $1163 \pm 307.39$  mg/dl,  $P<0.05$ ), and IgA ( $76.15 \pm 40.0$  vs.  $153.24 \pm 29.61$  mg/dl,  $P<0.01$ ). The IgM antibodies showed lower concentration in smoker's polycythemia and it was not statistically significant.

Complement ( $C_3, C_4$ ) showed also significantly ( $P<0.01$ ) lower concentration in smoker's polycythemia and it reached ( $66.63 \pm 29.25$  and  $10.13 \pm 7.44$  mg /dl respectively). It was apparent that there was significant increase ( $P<0.01$ ) in the percentage of positive cases for CRP and reached 100% in smoker's polycythemia than in control (no positive cases were observed) .

**Table 2: Comparisons in immunological parameters between smoker's polycythemia and control (non smoker)**

Immunological p.	Smoker's polycythemia	Control (non smoker)
IgG(mg/dl)	$864.75 \pm 159.51^*$	$1163 \pm 307.39$
IgA(mg/dl)	$76.15 \pm 40.0^{**}$	$153.24 \pm 29.61$
IgM(mg/dl)	$89.34 \pm 32.79$	$116.85 \pm 34.91$
C3 (mg/dl)	$66.63 \pm 29.25^{**}$	$130.48 \pm 33.33$
C4 (mg/dl)	$10.13 \pm 7.44^{**}$	$31.9 \pm 2.24$
CRP(%)	100	0

Mean  $\pm$  SD (standard deviation) ; \*  $P<0.05$  , \*\*  $P<0.01$

Table (3) Showed significant difference between smoker's polycythemia and control in serum TC ( $6.04 \pm 1.19$  vs.  $2.86 \pm 0.32$  mmol/L,  $P<0.01$ ), TG

( $1.55 \pm 0.33$  vs.  $0.93 \pm 0.42$  mmol/L,  $P<0.01$ ) and HDL-C ( $0.39 \pm 0.03$  vs.  $0.57 \pm 0.21$  (mmol / L,  $P<0.05$ ).

**Table 3 :Comparisons in biochemical Parameters between smoker's Polycythemia and control (non smoker)**

Biochemical parameters	Smoker's polycythemia	Control(non smoker)
Total cholesterol mmol/L	$6.04 \pm 1.19^{**}$	$2.86 \pm 0.32$
Triglyceride mmol/L	$1.55 \pm 0.33^{**}$	$0.93 \pm 0.42$
HDL- cholesterol mmol/L	$0.39 \pm 0.03^*$	$0.57 \pm 0.21$

HDL: high –density lipoprotein .

Mean  $\pm$  SD (standard deviation); \*  $P<0.05$  , \*\* $P<0.01$

## Discussion

In this study, the results indicated the highly effect of cigarette smoking on the hematological parameters, which may due to hypoxia as a result of increase carbon monoxide or other chemical constituents in cigarette smoking (10,11). In response to hypoxia, the hormone erythropoietin, secreted by the kidneys, stimulates the bone marrow to produce certain blood elements including WBCs, Pc and RBCs. The latter causes the increasing in concentrations of Hb and MCH, a component of all red blood cells(12).The elevated total and differential WBCs count found in smoker's is agreement with findings of other investigators (13,14), and there are several possible mechanisms of smoking. Induced leukocytosis, such as smoking -induced chronic inflammation(14), catecholamine release from the adrenal medulla(13), carboxy hemoglobin concentration that reflecting exposure to cigarette smoke(4). In addition elevated counts of monocytes which may be reflect increase production of these cells in smokers, that is thought to be the precursor of the pulmonary macrophage, and are found in larger numbers in the lungs of smokers than in non smokers(14). While found significant decrease in antibody serum concentrations of IgG and IgA, the reduced immunoglobulin concentrations indicate that cigarette smoking may be associated with the suppression of B- cell function and

## Reference

1. Tefferi, A. (2003) .Polycythemia vera :comprehensive review and clinical recommendations. Mayo. clin. Proc. ;78 :174-194.
2. Diehn, F. and Tefferi, A. (2001). Pruritus in polycythemia vera :prevalence ,laboratory correlates and management .Br. J. Haemat. ;115: 619-621
3. El-zayadi, A. R. (2006) .Heavy smoking and liver .world. J. Gastroenterol . ;12(38) :6098-6101.
4. El-zayadi, A. R.; Selim, O.; Hamdy, H. :El-Tawil, A. and Moustafa, H. (2002). Heavy cigarette smoking induces hypoxic polycythemia (erythrocytosis ) and hyper uricemia in chronic hepatitis C patients with revisal of clinical symptoms and laboratory parameters with therapeutic phlebotomy .Am. J. Gassteroenterol; 97:234-254.
5. Mancini, G.; Carbonana, A. O. and Herwans, J. (1965) . Immunochemical quantitation of antigen by single radial Immunodiffusion . Immunochem.:234-254.
6. Warworth, E. and Wadsworth ,Ch. (1984). A rapid slide test for the qualitative and quantitative determination of c- reactive protein in human serum .Clin. Chim. Acta.;138.
7. Richmon, N. (1973) . Cholesterol enzymatic end point method Clin. Chem. U.K. 19 :1350-1356.
8. Klin, Z. (1972). HDL. Cholesterol :chem.. U.K. Klin. Biochem., 10 :182.
9. Bucolo, G. and David, M. (1973). Liquid triglycerides enzymatic colorimetric test . Clin. Chem. 19:476.

immunoglobulin production and this may contribute to the increased susceptibility to infections in this population(15,16,17,18). About IgM concentration found markedly decrease, because IgM are characteristic of primary immune responses. On the other hand significal increased in the percentage of positive cases for CRP may be due to synthesized it from the liver as result of releasing pro inflammatory cytokines from the inflamed tissue. The physiological role of CRP is not fully understood; it was discovered by its ability to bind to pneumococcal C-polysaccharide, also found its ability to activate complement classical path way, that after tissue injury, CRP binds to damaged cells and, by activating complement, may contribute to the inflammatory response(19).This evidence may agreement with the decrease serum levels of circulating complement component C<sub>3</sub> and C<sub>4</sub>(table 2), which may be due to the localization of CRP in relation to deposition of activated complement in inflamed tissue ,and may help to narrow the differential diagnosis (20,21,22). About biochemical parameters results showed significal higher serum concentrations of TC and TG, those data are compatible with the previous studies by(23, 24) and ( 25).Leukocytosis and polycythemia in addition to low HDL-C and high TC with TG levels may play roles in the development of atherosclerosis in smokers.

10. Arcavi, L. and Beno, N. (2004) .Cigarette smoking and infection .Arch. Intern. Med; 164:2206 -2216.
11. Dye, J. A. and Adler, K. B. (1994) . Effects of cigarette smoke on epithelial cells of the respiratory tract. Thorax.49:825-834.
12. Van Eeden, S. F. and Hogg, J. (2000). The response of human bone marrow to chronic cigarette smoking .Eur. Respir. J.;15 :915-921.
13. Taylar, R. G.; Woodman, G. and Clarke, S. W.(1986). Plasma nicotine concentration and white blood cell count in smokers .Thorax. 41: 407-408.
14. Tell, G. S.; Grimm, R. H. ;Vellar, O. D. and Theorsen, L.(1985) .The relationship of while cell count, platelet count, and hematocrit to cigarette smoking in adolescents: The Oslo Youth Study. Circulation; 72(5):971-974.
15. Barbour, S. E. ; Nakashima, K. ;Zhang, J. B. ;Tangada, S. ; Hahn,C. L. ;Schenkein, H. A. and Tew, J. G. (1997) . Tobacco and smoking .environmental factors that modify the host response(immune system).and have an impact on . Periodontal health . Crit. Rev. Oral. Biol. Med.;8(4) :437 -460.
16. Gemmell, E. and Seymour, G. D. (2004) .Immunoregulatory content of Th1LTh2 cytokine profiles in periodontal disease Periodonto. D.;35:21-41.
17. Staines, N. A.; Brostoff, J. and Jamas, K. (1993). Introducing immunology 2<sup>nd</sup> ed. St. Louis.; London. Mosby.

18. Moszczynski, P. ; Zabinki, Z. ; Moszczynski, P. Jr.; Rutowski, J. ; Slowinski, S. and Tabarowski, Z. (2001) . Immunological findings in cigarette smokers. Toxicol. Lett. ;118: 121-127.
19. Levinson, W. and Jawetz, E. (2000). Medical microbiology and immunology: Examination and board review .6<sup>th</sup> ed. Lange. Med. Books / McGraw-Hill;New York:pp.582.
20. Anjaroos, S. P. and Data, M. R. (2005) . Complement and the kidney :what the nephrology needs to know in 2006?Oxford J. Med. NDT. 20(12):2613-2619).
21. Nauta, A. J. ; Castellano, G. and Xu, W. (2004) . Opsonization with C19 and mannose-binding lectin targets apoptotic cells to dendritic cells . J. Immunol. 173 :3044-3050.
22. Nauta, A. J. ;Ttouw, L. A. and Data, M. R. (2002). Direct Binding of C19 to apoptotic cells and cell blebs induces complement activation . Eur. J. Immunol.;32:1726-1736.
23. Tell, G. S. ;klepp, K. I. ;Vellar, O. D. and McAlister, A. (1984). Preventing the onset of cigarette smoking in Norwegian adolescent : The Oslo Youth. Study. Prev. Med. 13:265.
24. Sharma, S. B. ; Dwivedi, S. ; Prabhr, K. M.; Singh, g. ; Kumar, N. and Lai, M. K. (2005) . Coronary risk variables in young asymptomatic .Smokers. Indian.
25. Richardson, N. A. (1988). Upper airway complications of cigarette smoking. J. Allergy Clin. Immunol.; 81: 1032-1035.

## تقدير المعايير الدموية والمناعية والكيموحيوية في المرضى المصابين بفرط نمو كريات الدم الحمراء الثانوي

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( تاريخ الاستلام: ٦ / ٣ / ٢٠١١ ---- تاريخ القبول: ٢٧ / ٩ / ٢٠١١ )

### الملخص

صممت هذه الدراسة بهدف البحث عن العلاقة بين المعايير الدموية والمناعية والكيموحيوية وكثرة التدخين في المصابين بفرط نمو كريات الدم الحمراء الثانوي. ولأجل تحقيق ذلك فقد شملت الدراسة ٦٠ فرد والذين توزعوا على مجموعتين أساسيتين ضمت المجموعة الأولى المدخنين بكثرة والمصابين بفرط كريات الدم الحمراء الثانوي (٤٠ مدخناً). في حين شملت المجموعة الثانية الأصحاء غير المدخنين وغير المعرضين للتدخين (٢٠ شخصاً)، لقد أفرزت النتائج وجود زيادة معنوية في مستوى الهيموغلوبين، كمية الهيموغلوبين في كرية دم حمراء والتعداد الكلي لكريات الدم الحمراء والبييض والصفائح الدموية وتركيز الكولسترول الكلي والكليسيريدات الثلاثية ، ارتفع أيضاً معدل النسبة المئوية للحالات الموجبة للبروتين CRP ارتفاعاً معنوياً بلغت ١٠٠%. بينما كان هناك انخفاض معنوي في تركيز مستوى الكليبولينات المناعية IgG, IgA والتراكيز المصلية لبروتينات المتمم C<sub>3</sub>, C<sub>4</sub> في المدخنين بكثرة والمصابين بفرط كريات الدم الحمراء الثانوي عنها في مجموعة السيطرة .