The effect of sever glucose-6-phosphate dehydrogenase(G6PD) deficiency on the activity of white blood cells for a female medical students in Basrah University.

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ABSTRACT :

This study involved (57) female medical student, aged between (19-24) years, subdivided into two groups, control group that consist of (30) female with normal level of G6PD enzyme and case group that consist of (27) female with severe G6PD deficiency. Both groups have hemoglobin type AA, and white blood cells were estimated for them. We tried in this study to identify the effect of sever G6PD deficiency on the phagocytic activity of non isolated granulocytes from the whole blood, to mirror the in vivo stimulation of granulocytes and the effect of severe G6PD deficiency on their phagocytic activity. We demonstrate a statistically significant proportion ($P < 0.05$) between the granulocytes phagocytic activity and severe G6PD deficiency. This could form the basis for drug development in order to prevent or treat G6PD deficiency-related disease and thus unburden the public health system.

Key Words: G6PD, granulocyte, Phagocytosis.

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INTRODUCTION:

Glucose-6-phosphate dehydrogenase deficiency, is an x-linked recessive hereditary disease, characterized by abnormally low levels of glucose-6-phosphate dehydrogenase (G6PD)[1], a metabolic enzyme involved in the pentose phosphate pathway, especially important in red blood cell metabolism[2]. Individuals with the disease may exhibit non immune hemolytic anemia in response to a number of causes, most commonly, consumption of broad beans, exposure to certain medications or chemicals and infections[3]. Possible mechanisms for the sever deficiency of G6PD in erythrocytes and plymorphonuclear leucocytes(granulocytes), were investigated[4]. The granulocytes exhibit Phagocytosis, which is an essential function of immune system[5]. Actively phagocytizing granulocytes emit light or chemiluminescence's (CL) which has been shown to be linked to the oxidative activity of the phagocytizing polymorphonuclear leucocytes[6]. The production of reactive oxygen metabolites by granulocytes plays a key role in a host defense against invading microorganisms and foreign bodies[7]. The ability of granulocytes to kill bacterial organisms by a process of Phagocytosis respiratory burst is related, in part, to their capacity to generate several reactive oxygen species(ROS)[8]. These (ROS) include [super oxide, nitric oxide, hydrogen peroxide, hydroxyl radical and singlet oxygen][9].The term respiratory burst

refers to a coordinated series of metabolic events that takes place when phagocytes exposed to appropriate stimuli[10]. This group of events underlies all oxygen-dependent killing by phagocytes and a sharp increase in oxygen uptake occurs upon stimulation[11]. The potent (ROS) generated by phagocytes is capable of oxidizing ***luminol*** (chemiluminescence's indicator), and chemiluminescence's light bursts are produced[12]. This technique of luminol-amplified chemiluminescence is a sensitive system, permitting the use of less than 10^4 phagocytes per assay[13]. Luminol can react with the (ROS) generated during Phagocytosis to produce an excited intermediate state that emits light upon returning to the ground state[14]. Luminol-amplified chemiluminescence activity can be simplified by a formula:[*Luminol + ROS*$^{peroxide\ catalyst}$ *N2+amino-phthalate ion+Light*.].

AIM OF STUDY:

The aim of our work was to study CL of whole blood stimulated by Barium sulfate crystals($BaSO_4$) to evaluate the activity of granulocytes and their relation with G6PD deficiency. This could form the basis for drug development in order to prevent or treat the G6PD deficiency-related disease, and thus to unburden the public health system.

MATERIALS AND METHOD:

Preparation of blood samples:

Venous blood samples(0.8ml) were obtained from (57) female medical students, aged between (19-24) years from Medical College during the academic educational year 2007-2008. Investigations have been done to identify the type of hemoglobin by (hemoglobin electrophoresis method), WBC_s count and G6PD secrening test (fluorescent spot test) .They are subdivided into two groups,(control group) that consist of (30) healthy female with normal G6PD and type AA hemoglobin and (case group) that consist of (27)female identified as severely erythrocyte G6PD deficient(full expression) with type AA hemoglobin. Each sample of blood was mixed with(0.2ml) of 5% sodium citrate (Na-citrate, FLUKA-GUARANTEE) as anti coagulant in measuring vial, and then kept at 37C$^{\circ}$ until the start of the assay(usually CL was measured within 1 hr.) and the granulocytes were counted. The number of cells were estimated by using heamocytometer. Luminol solution was prepared by dissolving $1.13x10^{-2}$ M of luminal(5-amino-2,3-dihydro-1,4-phthalaziuedione) (Sigma chemical Co.) in 2ml of 0.2M NaOH (Raidel DeHaen), this stock solution was diluted up to 100ml with deionized water and kept prior to use. In order to activate granulocytes to burst, a medium of the following composition(mM) was used(CL inducer) : 165 sodium chloride, 15 Tris hydrochloric acid, 2.25 $BaSO_4$(Barium sulfate) (PH=8). $BaSO_4$ in this medium was in a suspended form.**(** Chemiluminescence easurement)**.**The reaction mixture consisted of 2ml CL inducer, 0.2ml NaOH and 0.2 luminol in a 5ml beaker. To this mixture 0.02ml whole blood was added and agitated to mix well before it was poured into the measuring cuvette of an ultra-high-sensitive photon counting system[14]. The temperature was kept at 37C$^{\circ}$ during the counting. CL was continuously recorded on a chart recorder, until the CL peaked and demonstrated a definite decline. The results of CL in the peak height curve were estimated The results analysis were performed with SPSS statistical

software version 10. ANOVA analysis of variance probability value of < 0.05 was considered to be statistically significant. All the measurements were estimated in mm peak height and related to the same number of cells i.e.(100cells) for the purpose of the comparism between the two groups.

RESULTS:

The results of granulocytes functional activity were express as mean ± SD comparism between the control group and the case group using ANOVA analysis of variance. P-value < 0.05 regarded a significant relation between G6PD deficiency and WBC activity (CL peak) as shown in table (1), fig. (1) and fig. (2).

DISCUSSION :

The polymorphonuclear granulocytes are the major defense against different types of infections. Non isolated granulocytes were tested for their phagocytic activity in whole blood to mirror the in vivo stimulation of granulocytes and the effect of G6PD deficiency on their phagocytic activity. In this study we demonstrated a statistically significant difference with a P-value (P < 0.05) between G6PD deficiency and the phagocytic activity of the granulocytes. While, there is no statistical difference in the number of WBC_s count between the two groups. In recent years, CL has emerged as an important tool in the assessment of the oxidative burst of granulocytes[15]. The exact nature of this CL is thought to be a result of the interaction of biologically active oxygen radicals and excitable substrates within the cell[16]. The technique involves the use of luminol to increase the amount of measurable light emitted due to liberations of oxygen metabolites during Phagocytosis[17].Glucose-6-phosphate dehydrogenase is the first enzyme in the pentose phosphate pathway[18], and the main intracellular source of reduced nicotidamineadenine nucleotide phosphate (NADPH)[19], involved in diverse physiological processes such as anti oxidant defense(for instance in the erythrocyte), endothelial growth modulation, erythropoyesis, vascularization and Phagocytosis[20]. The sever deficiency of this enzyme results in a reduction of (NADPH) generation[21], which results in a decrease in the productions of hydrogen peroxide(H_2O_2), nitric oxide(NO) and peroxide[22]. The bactericidal activity of the nutrophils depends primarily on free oxygen radicals released by the activation of (NADPH) oxidase[23]. Therefore, the nutrophil microbicide activity is altered in individuals with G6PD deficiency and likewise it's inflammatory response[24]. There was a deep defect in the respiratory explosion that accompanies the Phagocytosis of all myeloid cells(neutrophil, eosinophil, monocyte and macrophage)[25], lead to increase the susceptibility to recurrent bacterial infections. So, host defenses may be altered in G6PD deficiency and bacterial infections are more sever[26]. Alternatively, G6PD deficiency and infections might represent concomitant risk factors which lead to hospitalization during bacterial infections[27]. Although, the deficiency protects against malaria but was shown to worsen the clinical course after trauma[28]. The patient with G6PD deficiency that exposed to trauma have an aggravated inflammatory response and increased incidence of septic complications and or more profound alterations in leukocyte functions compaired with non deficient trauma patient[29].Patient with G6PD deficiency is associated with low level of reduced glutathione[30], increased DNA damage may be a result of deficient detoxification of reactive oxygen

species by glutathione and may ultimately account for the higher rate of apoptosis in G6PD deficient granulocytes[31].It is concluded that sever glucose-6-phosphate dehydrogenase (G6PD) deficiency is associated with granulocytes dysfunctions and increase the susceptibility to recurrent infections.

Table (1) CL peak activity/ 100 cell

No. of subjects		W.B.C$_s$. Activity* (mean ± SD)
control	30	11.1870±7.23685
case	27	7.4037±3.44886

Fig. (1) statistically significant difference with a P<0.05 (ANOVA analysis of variance).

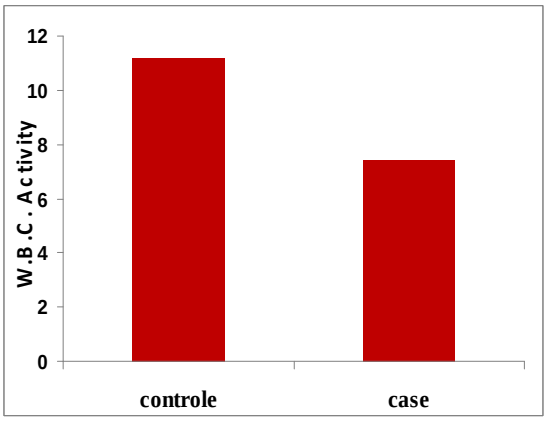
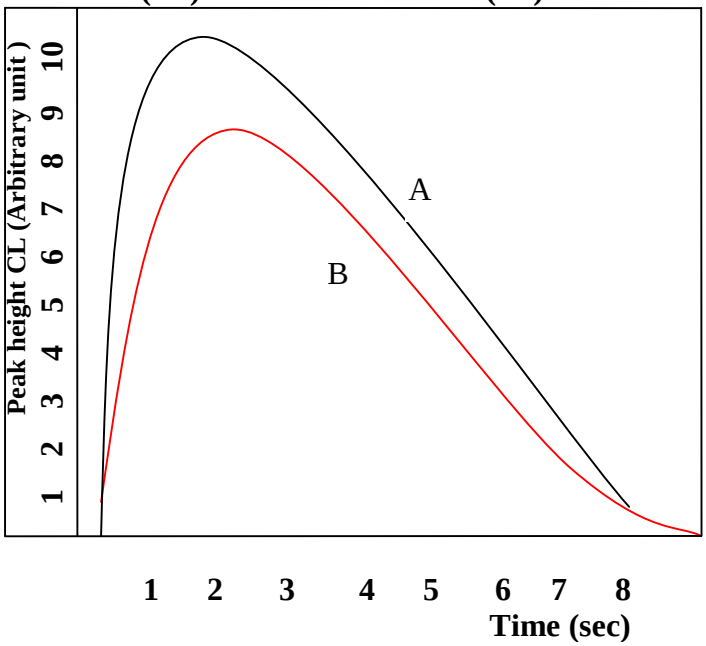


Fig.(2) :Patron of CL peak height of W.B.C activity in: normal (A)and G6PD deficient (B).



REFERENCES:

1. Kwesi Sackey MD. Hemolytic Anemia: Part 1. American Academy of Pediatrics. 1999; 20:152-159.

2. Ekremoglu M , Turkozkan N, Erdamar H, Kurt Y and Yaman H. Protective effect of taurnine on respiratory burst activity of polymorphonuclear leukocytes in endotoxemia. Amino Acids.2007; 32(3):413-417.

3. Allison AC. Observational, Hypothesis-Driven and Genomics Research Strategies for Analyzing Inherited Differences in Responses to Infectious Diseases. Public Health Genomics. 2009; 12: 41-52.

4. Kawai T, Akira S. Innate immune recognition of viral infection. Natural Immunology (February 2006); 7(2):131-7.

5. Kristina N, Jozef F, Katalin C, Katalin S and Lakatos S. Luminol- dependent chemiluminescence is related to the extracellularly released reactive oxygen intermediates in the case of rat nutophils activated by formyl-methiony-leucyl-phenylalanine. 2001; 31(4):277-285.

6. Lee S. Measurement of whole blood phagocyte chemiluminescence in a microtitreplate format. Clinical and Laboratory Haematology. 2008;14(3):231-237.

7. Gallin JI, Goldstein IM and Snyderman R. Inflammation: basic principles and clinical correlates. New York, Raven Press, 1992.

8. Gill GN. Text book of medicine,6thed. Philadelphia, WB Saunders Company, 1996.

9. Bagchi K , Puri S. Free radicals and antioxidants in health and disease. Eastern Mediterranean health journal.1998;4(2):350-60.

10. Bellavite P. The superoxide-forming enzymatic system of Phagocytes. Free radical biology and medicine. 1998;4(4):225-61
.
11. Dahlgren C. Chemiluminescence as following function of phagocytic cells. Photochemistry and Photobiology. 1991;2:427-42.

12. Van Dyke K and Castranova V. Cellular Chemiluminescence. Boca Raton, Florida, CRC Press, 1987.

13. Dahlgren C. Quantitative slot-blot chemiluminescence assay for determination of myeloperoxidase from human granulocytes. Analytical biochemistry. 1993;214(1):284-8.

14. Al-Hashimi HM and Mohammad FH. An ultra-high-sensitive photon counting system and it's application to biomedical measurements. Basrah journal of science. 1997;15(1):1-7.

15. Inga W, Judith J, Lother P and Peter U. Immunosenescence of polymorphonuclear Neutrophils. The Scientific World Journal. 2010;10:145-160.

16. Cheson BD, Christensen RL and Sperling R. The origin of the chemiluminescence of phagocytosing granulocytes. Journal Clin Invest. 1976;58:789-796.

17. Egger G, Elisabeth M, Hayn M and Judith S. Changes in polymorphonuclear leukocytes function of blood samples induced by storagtime, temperature and agitation. Journal of Immunological Methods. 1997;206(1-2):61-71.

18. Ajlaan SK, Al-Naama LM and Al-Naama MM. Correlation between normal glucose-6-phosphate dehydrogenase level and haematological parameters. Eastern Mediterranean Health Journal. 2000;6(2/3):391-395.

19. Corrons Vives JL, Feliu E, Pujades MA, Cardellach F, Rozman C, Carreras A, Jou JM, Vallespi MT and Zuazu FJ. Sever-glucose-6-phosphate dehydrogenase (G6PD) deficiency associated with chronic hemolytic anemia, granulocytes dysfunction, and increased susceptibility to infections: description of a new molecular variant(G6PD Barcelona). Blood.1982;59(2):428-434.

20. Javier Fernando Bonilla MD, Carolina M and Chuaire L. Glocose-6-phosphate dehydrogenase (G6PD). Response of the human erythrocyte and an other cells to the decrease in their activity. Colombia Medica. 2007; ISSN 1657-9534 Version online.

21. Tabbara KF, Sharara NA and Al-Momen AK. Toxoplasmosis in a group of glucose-6-phosphate dehydrogenase deficient patients. Saudi Medical Journal. 2001;22(4):330-332.

22. Tsai K. Impaired production of nitric oxide, superoxide, and hydrogen peroxide in glucose-6-phosphate-dehydrogenase-deficient granulocytes. Elsevier. 2009;436(3):411-414.

23. Roos D, Zwieten R, Wijnen JT and Gallego FG. Molecular Basis and Enzymatic Properties of Glucose-6-phosphate Dehydrogenase Volendam, Leading to Chronic Nonspherocytic Anemia, Granulocytes dysfunction, and increased Susceptibility to Infections. Blood. 1999;94(9):2955-2962.

24. Bruggen R, Bautista JM, Petropoulou T and De Boer M. Deletion of leucine 61 in glucose-6-phosphate dehydrogenase leads to chronic nonspherocytic anemia, granulocyte dysfunction, and increased susceptibility to infections. Blood.2002;100(3)1026-1030.

25. Clark M and Root RK. Glucose-6-phosphate deydrogenase deficiency a study of hospitalized patients in Iran. Yale Journal of biology and medicine.1979;52(2):169-179.

26. McNicholl JM, Downer MV, Udhayakumar V, Alper CA and Swerdlow DL. Host-Pathogen Interactions in Emerging and Re-emerging Infectious Disease: A Genomic Perspective of Tuberculosis, Malaria, human Immunodeficiency Virus infection, Hepatitis B and Cholera. Annual Review of public

Health. 2000;21`:15-46.
27. Ardati KO, Bajakian KM and Tabbara KS. Effect of Glucose-6-Phosphate Dehydrogenase Deficiency on Neutrophil Function. Acta Haematologica. 1997;97(4):211-215.

28. Jeanette W, Erika V, Edwin AD and Zoltan S. Gloucose-6-phosphate dehydrogenase deficiency and the inflammatory response to endotoxin and polymicrobial sepsis. Critical Care Medicine. 2007;35(2):510-518.

29. Jeanette W. Increased incidence of sepsis and altered monocyte functions in sever injured type A- glucose-6-phosphate dehydrogenase deficient African-American trauma patients. Critical Care Medicine .2001;29(4):728-736.

30. Reddy BM and Tripathy V. Present status of understanding on the G6PD deficiency and natural selection. Journal of Postgraduate Medicine.2007;53(3):193-202.

31. Efferth T, Fabry U and Osieka R. DNA damage and apoptosis in mononuclear cell, from G6PD deficient patients. Journal of Leukocyte Biology.2001;69:340-342.

دراسة تأثير النقص الحاد لنازعة هدروجين فسفات-٦-كلوكوز على فعالية الكريات البيضاء لطالبات كلية الطب/ جامعة البصرة

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الخلاصة:

هذه الدراسة شملت (٥٧) طالبة في كلية الطب ، تتراوح أعمارهم بين(١٩-٢٤) سنة، قسموا إلى مجموعتين إحصائيتين ، المجموعة الأولى (control) وتتكون من (٣٠) طالبة لهم مستوى طبيعي لنازعة هدروجين فسفات-٦-كلوكوز، والمجموعة الثانية (case) وتتكون من (٢٧) طالبة تعانى من نقص حاد لنازعة هدروجين فسفات-٦-كلوكوز. كلا المجموعتين لهم فئة دم (AA) . حاولنا في هذه الدراسة معرفة تأثير النقص الحاد لنازعة هدروجين فسفات-٦-كلوكوز على فعالية الكريات البيضاء غير المنفصلة من الدم ، وبرهنا على وجود تناسب رقمي هام($P< 0.05$) بين فعالية الالتقاف للكريات البيضاء والنقص الحاد لنازعة هدروجين فسفات -٦-كلوكوز. هذا ممكن أن يكون الأساس لتطوير الأدوية من اجل منع أو معالجة الأمراض المتعلقة بنقص نازعة هدروجين فسفات-٦-كلوكوز وذلك لإزاحة العبء عن النظام الصحي العام.

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