The effect of severe 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 students, 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 severe 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.

INTRODUCTION:
Glucose-6-phosphate dehydrogenase deficiency, is an x-linked recessive hereditary disease, characterized by abnormally low levels of glucose-6-phosphate dehydrogenase (G6PD), a metabolic enzyme involved in the pentose phosphate pathway, especially important in red blood cell metabolism. 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. Possible mechanisms for the severe deficiency of G6PD in erythrocytes and polymorphonuclear leucocytes (granulocytes) were investigated. The granulocytes exhibit Phagocytosis, which is an essential function of immune system. 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. The production of reactive oxygen metabolites by granulocytes plays a key role in a host defense against invading microorganisms and foreign bodies. 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). These (ROS) include [super oxide, nitric oxide, hydrogen peroxide, hydroxyl radical and singlet oxygen]. The term respiratory burst...
refers to a coordinated series of metabolic events that takes place when phagocytes exposed to appropriate stimuli. This group of events underlies all oxygen-dependent killing by phagocytes and a sharp increase in oxygen uptake occurs upon stimulation. The potent (ROS) generated by phagocytes is capable of oxidizing luminol (chemiluminescence's indicator), and chemiluminescence's light bursts are produced. This technique of luminol-amplified chemiluminescence is a sensitive system, permitting the use of less than $10^4$ phagocytes per assay. Luminol can react with the (ROS) generated during phagocytosis to produce an excited intermediate state that emits light upon returning to the ground state. Luminol-amplified chemiluminescence activity can be simplified by a formula: $[\text{Luminol} + \text{ROS } \text{peroxide catalyst} N_2+\text{amino-phthalate ion} + \text{Light}]$.

**AIM OF STUDY:**

The aim of our work was to study CL of whole blood stimulated by Barium sulfate crystals ($\text{BaSO}_4$) 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), WBCs count and G6PD screening 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 37°C 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 haemocytometer. Luminol solution was prepared by dissolving $1.3x10^{-2}$ M of luminal(5-amino-2,3-dihydro-1,4-phthalazinedione) (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 $\text{BaSO}_4$ (Barium sulfate) (PH=8). $\text{BaSO}_4$ in this medium was in a suspended form. 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. The temperature was kept at 37°C 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.,(100 cells) for the purpose of the comparison 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 WBCs count between the two groups. In recent years, CL has emerged as an important tool in the assessment of the oxidative burst of granulocytes. 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. The technique involves the use of luminol to increase the amount of measurable light emitted due to liberations of oxygen metabolites during Phagocytosis. Glucose-6-phosphate dehydrogenase is the first enzyme in the pentose phosphate pathway, and the main intracellular source of reduced nicotidamineadenine nucleotide phosphate (NADPH), involved in diverse physiological processes such as anti oxidant defense (for instance in the erythrocyte), endothelial growth modulation, erythropoiesis, vascularization and Phagocytosis. The sever deficiency of this enzyme results in a reduction of (NADPH) generation, which results in a decrease in the productions of hydrogen peroxide(H2O2), nitric oxide(NO) and peroxide. The bactericidal activity of the nutrophils depends primarily on free oxygen radicals released by the activation of (NADPH) oxidase. Therefore, the nutrophil microbicide activity is altered in individuals with G6PD deficiency and likewise it's inflammatory response. There was a deep defect in the respiratory explosion that accompanies the Phagocytosis of all myeloid cells(neutrophil, eosinophil, monocyte and macrophage), lead to increase the susceptibility to recurrent bacterial infections. So, host defenses may be altered in G6PD deficiency and bacterial infections are more severe. Alternatively, G6PD deficiency and infections might represent concomitant risk factors which lead to hospitalization during bacterial infections. Although, the deficiency protects against malaria but was shown to worsen the clinical course after trauma. The patient with G6PD deficiency that exposed to trauma have an aggravatd inflammatory response and increased incidence of septic complications and or more profound alterations in leukocyte functions compared with non deficient trauma patient. Patient with G6PD deficiency is associated with low level of reduced glutathione, 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. 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

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>W.B.C, Activity (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>30</td>
</tr>
<tr>
<td>case</td>
<td>27</td>
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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 ).
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دراسة تأثير النقص الحاد لنازعة هدروجين فسفات-6- كلوزوك
على فعالية الكريات البيضاء لطالبات كلية الطب/ جامعة البصرة

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الخلاصة:

هذه الدراسة شاملت (75) طالبة في كلية الطب، تتراوح أعمارهم بين (19-42) سنة، قسمنا إلى مجموعتين إحصائيتين. المجموعة الأولى (control) وتتكون من (30) طالبة لهم مستوى طبيعي لنازعة هدروجين فسفات-6-كلوزوك، والمجموعة الثانية (case) وتتكون من (47) طالبة تعاني من نقص حاد لنازعة هدروجين فسفات-6-كلوزوك. كلا المجموعتين لهم فئة دم (AA). حاولنا في هذه الدراسة معرفة تأثير النقص الحاد لنازعة هدروجين فسفات-6-كلوزوك على فعالية الكريات البيضاء غير المنفصلة من الدم، وبرناها على وجود تناسب رقمي هام (P<0.05) بين فعالية الالتهابل للكريات البيضاء والنقص الحاد لنازعة هدروجين فسفات-6-كلوزوك. هذا ممكن أن يكون الأساس لتطوير الأدوية من أجل منع أو معالجة الأمراض المتعلقة بنقص نازعة هدروجين فسفات-6-كلوزوك وذلك لإزالة العباء عن النظام الصحي العام.

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