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In-vitro study the effects of sickle cell disease on phagocytic activity of white blood cells among adult male in Basra city

دراسة مختبريه لمعرفة تأثير فقر الدم المنجلى على فعالية الالتهام الخلوي لكريات الدم البيضاء للذكور البالغين في البصرة

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Abstract:

Background: sickle cell disease (SCD) is an inherited disorder of hemoglobin synthesis that is associated with a significant morbidity and mortality. The patho physiology of the disease involves abnormalities not just in red blood cells but also white blood cells (leukocytes) functions.

Objective: this study was tried to identify the effect of sickle cell disease SCD on the phagocytic activity of non- isolated leukocytes from the whole blood, to mirror the *in vivo* stimulation of leukocytes and the effect of SCD on their phagocytic activity.

Materials and methods: this study involved (56) adult male, aged (18-30) years, subdivided into two groups; case group that consist of (26) patient with sickle hemoglobin phenotype (SS) in a steady state at the time of the study and control group that consist of (30) person with normal hemoglobin phenotype (AA). Hemoglobin electrophoresis and leukocytes count were estimated for both groups. The samples of blood were prepared for chemiluminescence (CL) measurements. Luminol was used to increase the amount of measurable light emitted due to liberation of oxygen metabolites during phagocytosis.

Results: Show a significant decrease in the phagocytic activity of SCD patients ($P < 0.0001$) in comparison with normal volunteers.

Conclusion: Sickle cell disease confers an increased susceptibility to infections due to decrease the phagocytic activity of stickler leukocytes, this could form the basis for drugs development in order to prevent or treat SCD- related infections and thus unburden the public health system.

Key Words: SCD, leukocytes, phagocytosis.

الخلاصة:

فقر الدم المنجلى هو مرض وراثي ارتكاسى يتولد نتيجة حدوث تغير في بروتين خضاب الدم (الهيموغلوبين) مما يسهم في تزايد ظواهر المرض والوفيات للأشخاص المصابين به. هذه الدراسة شملت (56) شخص من الذكور البالغين، تتراوح أعمارهم بين (18-30) سنه، قسموا إلى مجموعتين إحصائيتين،ألمجموعه الأولى وتتكون من (26) شخص يعاني من فقر الدم المنجلى وهم في حاله مستقره عند بدء الدراسة، إما ألمجموعه الثانية فتتكون من (30) شخص طبيعي الهيموغلوبين فئة (AA) ولقد أجريت التحاليل لمعرفة نوع الهيموغلوبين و عدد الكريات البيضاء لكلا المجموعتين . حاولنا في هذه الدراسة معرفة تأثير فقر الدم المنجلى على فعالية الكريات البيضاء غير المعزولة من عينات الدم بطريقة التألق الكيمياوى، حيث تم استعمال اللومينول لغرض زيادة الضوء المنبعث من تحرر جذور الأوكسجين الحرة الناتجة من عملية الالتهام.وبرهنا على وجود تناسب رقمي هام ($P\leq 0.05$) في نقص فعالية الالتفاف للكريات البيضاء لمرضى فقر الدم المنجلى مقارنة بالأشخاص الأصحاء. هذا ممكن إن يكون الأساس لتطوير الادويه من اجل منع أو معالجة الالتهابات المرافقة والمتعلقة بفقر الدم المنجلى وذلك لإزاحة العبء عن النظام الصحي العام.

Introduction:

Sickle cell disease is a genetic abnormality involving the hemoglobin.[1]Patients present with a wide spectrum of disorders of a single- point mutation in which thymine substitute for adenine, thereby encoding valine instead of glutamine in the sixth position of beta chain.[2] Hemoglobin (S)

causing by the substitution causes polymerization of hemoglobin and erythrocytes sickling on exposure to hypoxia.[3] People with SCD have red blood cells that contain sickle hemoglobin (S) instead of normal hemoglobin(A) and they inherit two genes of sickle hemoglobin (SS) homozygous from their parents.[4] The pathophysiology of SCD includes dysfunction of erythrocytes and leukocytes.[5] The polymorphonuclear leukocytes cells (Neutrophil and monocyte) exhibit phagocytosis, which is essential function of immune system.[6] Actively phagocytizing leukocytes emit light or chemiluminescence s (CL) which has been shown to be linked to the oxidative activity of the phagocytizing polymorphonuclear leukocytes.[7] The process of phagocytosis refer to the ability of leukocytes to destroy the bacterial organisms through 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 take place when phagocytes exposed to appropriate stimuli.[10] The potent (ROS) generated by phagocytes is capable of oxidizing *luminol* (chemiluminescence s indicator) and chemiluminescence s light bursts are produced.[11] The formula: [*luminal + ROS*$^{peroxide\ catalyst}$ *N2 + amino-phthalate ion + light*] usually used to simplify the activity of Luminol - amplified chemiluminescence. This sensitive system procedure of luminal - amplified chemiluminescence permitting the use of less than 10^4 phagocytes per assay.[12] The aim of the study is to identify the CL of whole blood stimulated by barium sulfate crystals ($BaSO_4$) to clarified the activity of white blood cells and their relation with SCD. This could form the basis for drug development in order to prevent or treat the SCD – related infections and thus to unburden the public health system.

Materials and Method:

A case – control study was conducted amongst (56) male aged between (18-30) years. They are subdivided into two groups; case group that consist of (26) patients who attending the sickle cell clinics in Basra city at steady state of homozygous sickle cell and (30) subjects with hemoglobin phenotype (AA) served as control group.

Investigations have been done to identify the type of hemoglobin by (hemoglobin electrophoresis method) and estimation of leukocytes count for both groups.

Venous blood samples (0.8ml) were obtained from the two groups, each sample of blood was mixed with (0.2ml) of 5% sodium citrate as anti coagulant in measuring vial and then kept at 37C° until the start of the assay (usually CL was measured within 1 hour) and the leukocytes were counted by using hemocytometer. Luminol solution was prepared by dissolving [1.13×10^2 M of luminal (5-amino-2,3-dihydro-1,4-phthalaziuedione] (Sigma chemical Co.) in (2ml of 0.2M NaOH). This stock solusion was diluted up to (100ml) with deionized water and kept prior to use. In order to activate leukocytes to burst, a medium of the following composition (mM) was used (CL inducer): [165 sodium chloride, 15 tri hydrochloric acid, 2.25 $BaSO_4$ (Barium sulfate in a suspended form) the medium PH=8. 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 and the temperature was kept at ($37C^0$ during the counting. CL was continuously recorded on a chart recorder, until the CL peaked and demonstrated a definite decline. 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 comparison between the two groups.[13]

Statistical analysis:

The results analysis were performed with SPSS statistical software version 10 and expressed as mean ± SD comparison between the control group and case group using independent – samples T-test. Probability value of (<0.05) was considered to be statistically significant relation between SCD and leukocytes phagocytic activity.

Results:

Non isolated leukocytes were tested for their phgocytic activity in whole blood to mirror the *in - vivo* stimulation of leukocytes and the effect of SCD on their phagocytic activity.

In this study we demonstrated a statistically significant decrease in leukocytes phagocytic activity of SCD patients with a P value ($P<0.05$) in comparison with control group as shown in table (1). The difference in the number of leukocytes count between SCD patients and control group is considered to be extremely statistically significant increase, P value($P<0.0001$)as shown in table(2).

Table (1) chemiluminescence peak activity/100 cell

No. of volunteers		WBC_s Activity (mean ± SD)
control	30	12.1780±7.236
case	26	*8.5103±3.447

t=2.361 p-value=0.0219

*statistically significant decrease in CL activity in case group

Table (2) White blood cells count for control and case groups

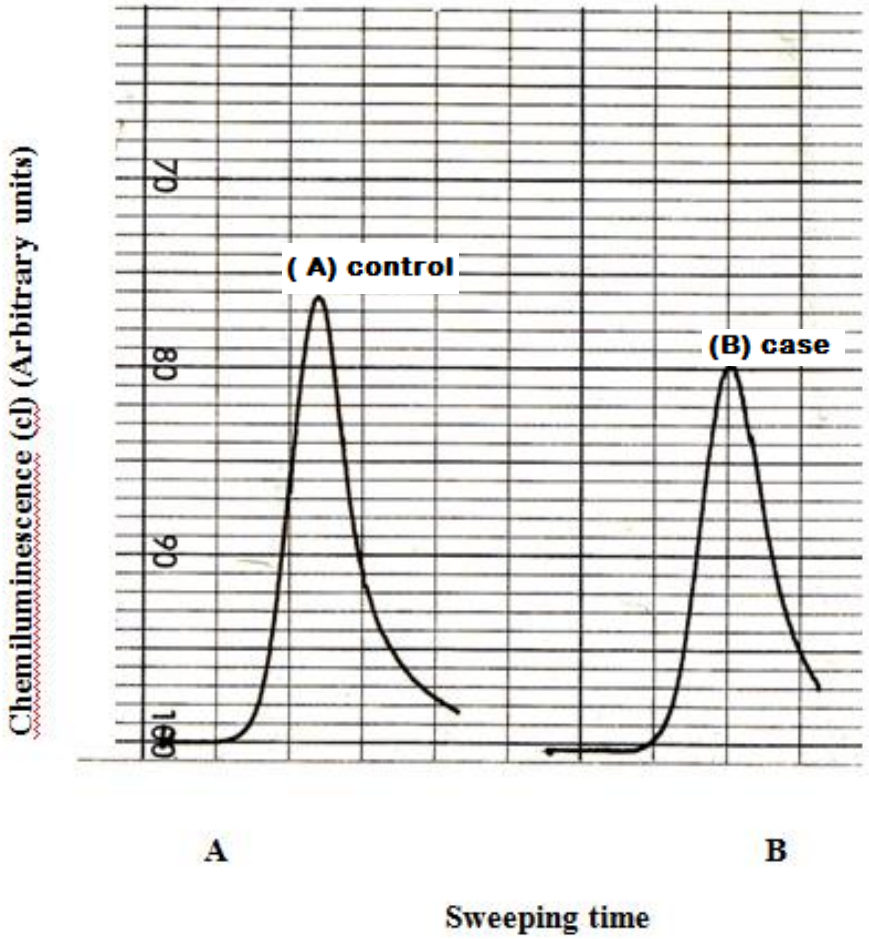
No. of volunteers		WBC_s count (mean ±SD)
control	30	6296 ±1489.54
case	26	**12896 ± 2441.05

t=12.393 p-value < 0.0001

**significant increase of WBC count in case group

Fig. (1) Patron of chemiluminescence peak height of white blood cells activity.

(A) control (B) case



Discussion:

Sickle cell disease (SCD) represents a spectrum of inherited hemoglobin disorders,[14] in which the mutation affects both erythrocytes and leukocytes functions.[15] Therefore, the disease usually associated with the susceptibility to infections despite the high leukocytes count.[16]And there is a fact that the severity of the disease increases with leukocytes count.[17] Accordingly, leukocytes contribute to SCD by adhering to blood vessel walls and obstructing the lumen, aggregating with other blood cells with more effective blockage of the lumen and causing tissue damage and inflammatory reactions.[18]The morbidity in SCD attributed to the bacterial infections.[19] The defense against bacteria depend mainly on normal neutrophil functions , it seemed that patient with SCD susceptibility to infections may result from several factors.[20] Primarily their ability to produce infection fighting antibodies is reduced and their leukocytes do not respond normally to invading germs[21]. The patient's capacity to target and destroy infected cells is impaired also their spleen are usually damaged by the disease.[22] Moreover, neutrophils and monocytes in SCD patients were significantly different from those in normal subjects in the areas of weaker phagocytosis, fewer ingested bacteria and reduced burst formation[23]. Recently it was found that the tufitsin phagocytic activity was decreased patient with SCD[24].

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