In vitro Age- dependent Leukocytes within whole blood activity among healthy adult men in Basrah measured by chemiluminescence.

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Abstract:

All immune cells are affected by aging, a fact contributes to the high susceptibility to infections and increased morbidity and mortality observed in the elderly. Non- isolated granulocytes were tested for their phagocytic activity in whole blood in vitro to mirror the in vivo stimulation of granulocytes and the effect of age progress on their phagocytic activity. Blood samples were obtained from(420) healthy adult men with different age groups from (16– 60) year. These samples 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. Chemiluminescence was continuously recorded on a chart recorder, and all the measurements were estimated in mm peak height and related to the same number of cells i.e. (100 cell) , for the purpose of the comparison between age groups. In this study a significant difference was demonstrated ($P< 0.05$) between leukocytes phagocytic activity with different age groups.

Introduction

Polymorphnuclear leukocytes(granulocytes) are the most predominant cellular component of the innate immune system in human being and produce an array of potent cytotoxic molecules(Kawai and Akira 2006) They exhibit phagocytosis, which is an essential function of immune system (Kristina *et al.* 2001). Actively phagocytizing granulocytes emit light or chemiluminescence (CL) which has been shown to be linked to the oxidative activity of the phagocytizing polymorphonuclear leukocytes(Lee 2008). The production of reactive oxygen metabolites by granulocytes plays a key role in a host defense against invading microorganisms and foreign bodies-(Gallin *et al.* 1992). The ability of granulocytes to kill bacterial organisms by the process of phagocytosis respiratory burst is related, in part, to their capacity to generate several reactive oxygen species (ROS) (Gill 1996). These (ROS) include: super oxide, hydrogen peroxide, hydroxy-radical and singlet oxygen(Bagchi and Puri 1998). The term respiretory burst refers to a coordinated series of metabolic events that take place, when phagocytes are exposed to appropriate stimuli(Bellavite 1988). This group of events underlies all oxygendependent killing by phagocytes and the sharp increase in oxygen uptake occuring upon stimulition(Dahlgren 1996). The potent (ROS) generated by phagocytes are capable of oxidizing ***luminol*** (chemiluminescence's indicator), and chemiluminescence light bursts are produced(Dahlgren 1993). This technique of ***luminolamplified chemiluminescence*** is a sensitive sys-tem, permitting the use of less than 10^4 phagocytes per assay(AlHashimi and Mohammed 1997). Luminol can react with the (ROS) generated during phagocytosis to produce an excited intermediate state that emits light upon returning to the ground state(Eggeret *et al.*-1997). Luminolamplified chemiluminescence act-

iveity can be simplified by a formula:

Luminol + ROS $\xrightarrow{\text{peroxide catalyst}}$ **N_2+amino-phthalate ion +light**(Cheson *et al.* 1976).

Aim of study:

The aim of this work is to study CL of whole blood stimulated by bar-ium sulfate crystals ($BaSO_4$) to evaluate the in vitro activity of gran-ulocytes and their correlations with human age.

Materials and Methods:

Preparation of blood samples:

Venous blood samples(0.8ml) each were obtained from(420) apparently healthy men (who were disease free at the time of doing the experiment), aged between (16-60) years. Each sample was mixed with (0.2ml) of (3.2%) sod-ium citrate (Nacitrate, FLUKA) as anticoagulant in a measuring vial, and then kept at 37C° until the start of the assay(usually CL was meas-ured within 1hr.). Total leukocyte count was done for all blood sam-ples and mean number was calcu-lated and recorded as(8000 cell/ml) of blood, so(100 cells) are included within (0.012ml) of who-le blood counted by a haemoc-ytometer (M× 200/9)(Follin 1992). Luminol solution was prepared by dissolving 1.13 x 10^{-2}M of lumin-ol (5-amino-2,3-dihydro-1,4-ph-thalaziuedione) (Sigma chemical Co.) in 2ml of 0.2M NaOH (Ria-del DeHaen), this stock solution was diluted up to(100ml) with deionized water and kept prior to use.In order to activate the leukocytes within the whole blood to burst, a medium of the following compositions (mM) was used (CL inducer) : (165mM)

sodium chloride, (15mM) Tris hydrochloric acid,(2.25mM) $BaSO_4$ (Barium sulfate) (PH=8). $BaSO_4$ in this medium was in a suspended form(Inga *et al.* 2110). The age group from (16-20) years was considered as a control (c) to compare between different age groups (t_1, t_2,…).

Chemiluminescence Measurement:

The reaction mixture consisted of ($2ml_s$) CL inducer, (0.2ml) NaOH and(0.2ml) luminol in a(5ml) beaker. To this mixture(0.012ml) whole blood containing(100) leukocytes added and agitated to mix well before it was poured into the measuring cuvette of an ultrahigh-sensitive photon counting system **(**Fulop *et al.* 2009) . The temperature was kept at 37C° during the coun-ting. 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. All the measurements were estima-ted in(mm)peak height and related to the same number of cells i.e. (100 cell) for the purpose of the comp-arison between age groups(Essellier *et al.* 1985).

Statistical analysis:

The results were analysed using (SPSS) statistical software version 10.A 2-taild probability value of (< 0.05) was considered to be statistically significant. Results of leukocytes functional activity were expressed as **(mean±SD)** ,and a comparison between control (c) and test(t) age groups using (t-test) was done. ($P<0.05$) was regarded as significant difference between age groups and leukocytes activity chemiluminescence(CL) peaks were shown in table(1) and fig.(1).

Results:

Table(1), shows mean values of chemiluminescence readings of different age groups. It is clear that mean chemiluminesce values decreased significantly between each two

groups of ages , and it is decreased gradually as age increased (P<0.05). This means that older men's leukocytes have less phagocytic activity than those of younger men. This is clear also from the activity inhibition of leukocytes when the inhibition increased due to older age.

Table (1): CL peak activity/ 100 cell

Age groups		No.	Mean	SD	Activity inhibition %
C	16-20	208	9.0105.**A**	3.555	0
T1	21-30	76	8.8481.**B**	4.0515	-1.8023
T2	31-40	84	8.5014.**C**	3.5295	-5.6500
T3	41-50	36	8.4892.**D**	3.378	-5.7854
T4	51-60	16	8.1592.**E**	3.489	-9.4478
Total		420	8.5372	3.5276	

* The difference is significant with (P< 0.05) (2-tailed).

* Activity inhibition % =((t-c)/c)×100.[13]

***Different capital letters between age groups means significant(P<0.05).**

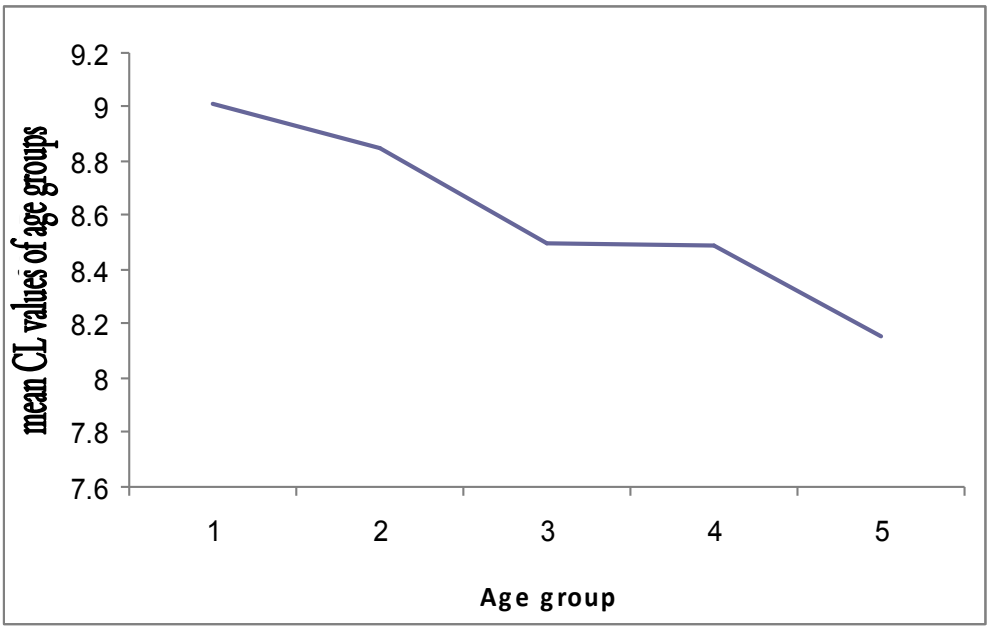

Fig.(1): The relationship between age groups and mean CL values of age groups

Discussion:

Non -isolated granulocytes were tested for their phagocytic activity in whole blood of different age groups in vitro to mirror the in vivo stimulation of granulocytes and to demonstrate any effect of age progress on their phagocytic activity. The immune response weakens during aging, certain functions of the innate immune system which build the line of defense against pathogenic microorganismms, are altered with aging(Essellier *et al.* 1985), resulting in increased incidence of inflammatory diseases and susceptibility to infections (Nagel *et al.* 1986) .There was no data available in neighboring countries dealing with phagocytic functional activity in different age groups for this study to compare with . In this study, healthy elderly humans, were included because comprromised functioning of innate immune responses might contribute to increased bacterial infections in them.

A significant decline in leukocytes phagocytic activity was demonstrated with age progress. Those results are agreed with Meszaros *et al.* 1999 & Srinivasan *et al.* 2005, who found a decrease in leukocytes activity with advanced age, and Peavey *et al.* 1985 who demonstrated that granulocytes functions such as : phagocytosis, generation of reactive oxygen species(ROS), intracellular killing, degranulations and possibly chemotaxis are changed in elderly persons(Peavery *et al.* 1985). The decline in leukocytes phagocytic activity in elderly persons was also in agreement with Lun et al 2000& Chandra 1997, who showed a functional decline of macrophages and granulocytes in aging as evidenced by their diminished phagocytic activity and impairment of superoxide generation. Therefore, there is no doubt among researchers that immune functions change with age, both humeral and cell mediated immune system, loss some of their ability to battle a variety of exogenous pathogens(Langly and Carrington 2006).

Conclusion:

Aging process is associated with an in vitro reduction in the leukocytes phagocytic activities. This may contribute to the high susceptibility to infections and increased morbidity observed in the elderly.

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دراسة تأثير التقدم في السن على نشاط الكريات الدموية البيضاء ضمن الدم الكلى في عينة من الرجال الأصحاء في البصرة مقاسه بطريقة التألق الكيمياوى خارج الجسم الحي

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الخلاصة:

تتأثر جميع خلايا المناعة بالتقدم في السن، مما قد يسهم في تفسيرا لقابليه العالية للعدوى، وتزايد ظواهر المرض والاعتلال التي تلاحظ في الكبر. ولغرض دراسة فعالية الكريات البيضاء المتعادلة في التقاف البكتيريا والأجسام الضارة، ومدى تأثرها بتقدم السن. تم إجراء الاختبار على كريات الدم البيضاء غير المعزولة من عينات دم مأخوذة من (٤٢٠) رجل بالغ سليم ، تتراوح أعمارهم (١٦-٦٠) حيث تم استعمال مادة اللومينول لغرض زيادة الضوء المنبعث من تحرر جذور الأوكسجين الحرة الناتجة عن عملية الالتقاف. وقد تم تسجيل الفعالية باستمرار على مسجل مخططات، وكل القياسات قد قيست بالملي متر ارتفاع القمة. تم احتساب الفعالية الحيوية مقدرة بارتفاع القمة إلى نفس الرقم من الخلايا إلى (١٠٠) خلية من اجل المقارنة بين كل مجموعة من المجاميع العمرية المختلفة. وقد وجد ارتباط معنوي بين فعالية الكريات البيض المتعادلة مع تقدم العمر($p<0.05$).