Study of phagocytosis activity in tuberculosis patients
دراسة فعالية البلعمة الخلوية لدى المصابين بالتدرن

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الخلاصة

اجريت هذه الدراسة خلال المدة من شهر كانون الاول 2008 ولغاية شهر حزيران 2009 للتعرف على بعض جوانب الاستجابة المناعية غير المتخصصة تجاه الاصابة بالـ Mycobacterium tuberculosis ، حيث بلغ معدل الخلايا الفعالة في بلعمة الخميرة المقتولة بعد 15 دقيقة لمجموعة السيطرة (40.25%) , وارتفع هذا المعدل لدى المصابين اصابة اولية بالتدرن ليصل الى (61.94) ، وكان هذا الارتفاع معنوياً عند المقارنة بمجموعة السيطرة ومجموعة المصابين بالتدرن اصابة ثانوية (45.09%) . واستمر معدل النسبة المئوية لمعامل البلعمة الخلوية بالارتفاع بعد مرور 30 ، 45 دقيقة لدى مرضى الاصابة الاولية ليصل الى (64.06% , 66.24%) على التوالي مقارنة بمجموعة السيطرة (43.75% , 42.95%) على التوالي وكان هذا الارتفاع معنوياً ، في حين سجل اعلى ارتفاع لهذا المعدل لدى مرضى الاصابة الاولية (68.53%) بعد مرور 60 دقيقة مقارنة مع (47.75%,41.73%) على التوالي للوقت نفسه لعينة السيطرة ومرضى الاصابة الثانوية وكان هذا الارتفاع معنوياً ايضاً .

Abstract

This study was carried out during the period from December 2008 until June 2009 for detection and evaluated the non-specific immune response against Mycobacterium tuberculosis infection , the average number of active cell in phagocytosis killing yeast after 15 minutes for control group (%40.25),this average was increased in primary tuberculosis patients to reach (%61.64) . Thise increase was significant as compared the control group and secondary tuberculosis patients (%45.09).This average continue in increase after 30,45 minutes in the same primary tuberculosis patients to reach (%64.06 , %66.24) respectively as compared with the control group (%43.75, %42.95) respectively and those increase was significant . Those average recorder highest increase in primary infection patients (%68.53) after 60 minutes as compared with (%47.75 , %41.73) for the same time of control sample and secondary patients and those increase was significant also .

Introduction

Tuberculosis was endemic in animals in the Paleolithic period, long before it ever affected humans ,this disease, also known as *consumption* , has been known in all ages and climates . The pathogenesis of tuberculosis caused by organisms belonging to Mycobacterium tuberculosis complex . Inhalation of a single viable organisms has been shown to lead to infection , although close contact is usually necessary for acquisition of infection with regard to M. tuberculosis, 15% to 20% of persons who become infected develop disease . Disease usually occurs some years after the initial infections when the patients immune system breaks down for some reason other than the presence of tubercle bacilli within the lung . In a small percentage of infected hosts , the disease becomes systematic , affecting a variety of organs (1) . The production and development of lesions and their healing and progression are determined chiefly by the number of mycobacteria in inoculum and their subsequent multiplication, and the resistance and hypersensitivity of the host . There are two principle lesions ,first is educative type – this consists of an acute inflammatory reaction , with edema fluid, polymorphonuclear leukocytes , and, later, monocytes around the

tubercle bacilli,the second is productive type- when fully developed this lesion a chronic granuloma , consists of three zones : a central area of large , multinucleated giant cells containing tubercle bacilli , amid zone of pale epithelioid cells , often arranged radically ,and aperipheral zone of fibroblasts,lymphocytes and monocytes(2) .

Pathogenic mycobacteria , in particular M. tuberculosis , the causative agent of tuberculosis , have the remarkable capacity to circumvent destruction within one of the most hostile cell types of a vertebrate host,the macrophage (3) . Mycobacterium tuberculosis (MTB) is a facultative intracellular pathogen with which over a billion people have been infected and 3 million people die annually . The bacterium induces vigorous immune responses , yet evades host immunity persisting within phagosomes of the infected macrophages . Thus , it is necessary to delineate that the virulence – related intracellular survival mechanism and the host immune responses to eradicate M. tuberculosis on the molecular basis(4) . Phagocytosed M. tuberculosis either multiply inside the endocytic compartment of mononuclear phagocytes or they are destroyed by the host cell . Due to this macrophage shelter(ab) used by Mycobacterium tuberculosis is controlled by the cellular immune response protection against mycobacteria depends on alpha/beta T cells expressing the CD4 or CD8 phenotype (5).

The macrophage is paradigmatic cell regard to M. tuberculosis infection . Indeed , alveolar macrophage have been shown to play an essential role in the elimination of particle that enter the organism through the airways ; and have long been considered the first cell population to interact with the tubercle bacillus . More macrophages are recruited afterwards from the blood stream , and are in charge of maintaining the infection in the host (6) . Entry of mycobacteria into phagocytic cell can occur through binding to multiple receptors , all leading to the delivery of the bacilli into macrophage phagosomes . Although the precise receptor that mediates mycobacterial uptake in vivo is yet to be established , multiple molecules have been shown to trigger phagocytosis in vitro (7) . The success of pathogenic mycobecteria is largely attributed to their capacity to avoid destruction within host immune cells , in particular macrophages .

Mycrobacteria are efficiently internalized into macrophage phagosomes by many different receptors . However , in contrast to the normal course of events during which the phagocytosed cargo is shuttled to lysosomes . In so doing , these pathogens manage to circumvent immediate destruction , enabling them to establish a niche inside the macrophage , where they can survive and even replicate . In addition , it is becoming clear that in order to persist within this host cell , pathogenic mycobacteria must be able to prevent the activation of macrophage (3) . In the experimental TB model , the protective response has a distinct type of cytokine pattern , as demonstrated by manipulation of the immunes system through genetic knockout or the administration of specific monoclonal antibodies (8,9,10) . The same cytokine pattern seems to be protective in humans because children with defective receptors for IFN-Y or IL-12 are susceptible to mycobacterial disease (11,12).

Material and Methods

Primary infection is paucibacillary,practically non-contagious,difficult to diagnose,and of variable severity, in seriously immunodepressed patient,but also in individuals with IFN-γ or IL-12 receptor deficiency,it can develop into a disseminated form,which is sometimes fatal(13) Secondary infection means that the infection can progress after the development of an adequate specific immune response.This infection episode can develope in tow ways: by inhalation of new bacilli or by reactivation of the primary focus(14).Twenty eight patients were selected presented with primary and secondary infection of TB according to the diagnosis of clinicians, the age of the patients ranges from 5-71 years , additional group(control group) of 20 healthy persons subjected to TB examination was also taken, persons with recent history of TB infection and with any sign and symptom of TB infection were excluded, blood was drawn from each patient (preoperatively) by

vein –puncture and was collected separately in plane tube with heparin and used this blood for phagocytosis examination.

• Phagocytosis of killed yeast .

Half of milliliters from the collected blood was put in plane tube, then added for it o.o5 milliliters from killed yeast suspension which prepared by soluble 10 grams of *Saccharomyces ceverisiae* yeast made from Turkian pakamaya company in 150 milliliters of normal saline and put the suspension in water bath for 60 minutes , then this suspension was filtered after it's cooling , the cells were counted , and concentration was controlled to 1×10^7 cells/ml^3.

Oh point two of milliliters of Hank's balanced salt solution was added , and after mixed these components well inocubated at 37c° and phagocytosis was studied for four period (15,30,45,60) minutes , and after each period prepare blood film by spread technique (the slides were staind with leishman stain before examination) and phagocytosis coefficient was calculated as follow (15).

$$\text{Phagocytosis coefficient} = \frac{\text{Number of phagocytosis cells for yeast cells}}{\text{Total number of cells}} \times 100$$

Results and discussion

The tables 1,2,3,4 demonstrated that there were a significant changes in the phagocytosis percentage of patients with primary TB infection in comparison with healthy controls patients and patients with secondary TB infection after(15,30,45,60 minutes).

The polynuclear cells particularly neutrophil consist the essential cellular arm in non specific immunity from their main role in phagocytosis and evaluated this activity through different periods (15,30,45,60 minutes) due to knowledge influence of time factor in phagocytosis (15) .

The results of study were in agreement with investigators (16) who cited that phagocytosis activity was remained constant with the time passing and phagocytes ability remained high against pathogens while the tables 1,2,3,4 demonstrated that there were no significant changes in the phagocytosis percentage of patients with secondary TB infection in comparison with healthy controls and these results were provide that the secondary infection (reactivation type) is usually caused by tubercle bacilli that have survived in the primary lesion , and these differences between primary and reinfection are attributed to 1- resistance 2- hypersensitivity induced by the first infection of the host with tubercle bacilli (2) . The results in the same tables above revealed that patients with primary TB infection have a higher values of mean phagocytosis percentage (61.641 ± 8.004, 64.06 ± 9.01, 66.24 ± 6.19, 68.53 ± 6.02) respectively with significant differences ($P<0.01$) compared to that of healthy controls patients(40.25 ± 13.97 , 43.75 ± 14.1, 42.95 ± 9.4 , 47.75 ± 14.04) respectively and also have a significant differences ($P<0.01$) when compared to that of patients with secondary TB infection (45.09 ± 12.57 , 43.27 ± 12.14 , 45.36 ± 14.07 , 41.73 ± 12.28) respectively which don't have a significant differences when compared with healthy controls .

The elevation in phagocytosis percentage cited in the present study and the other concordant studies can be attributed to the cytokine that is produced from NK cells and dendritic cells at the early period of infection strongly induces macrophage activation (4) .

The results of the present study were in agreement with(17) who cited that the main route of entry of the Mycobacterium tuberculosis is the respiratory route , alveolar macrophages are the important cell types , which combat the pathogen and binding of M.tuberculosis to macrophages via surface receptors .

On the other hand (2) explained that the host unless die during the first infection with tubercle bacilli , acertain resistance is acquired and there is an increased capacity to localize tuberele bacilli , retard their multiplication , limit their spread , and reduce lymphatic dissemination . This can be attributed to the development of cellular immunity during the initial infection , with evident ability of mononuclear phagocytes to limit the multiplication of ingested organisms and even destroy them .

The surviving bacilli exist in a latent state and can become reactivated to develope active disease (18)

Boom, *et al* showed that M.tuberculosis has evolved mechanisms to block immune responses and the relative importance of these blocking mechanisms likely depends on the stage of M.tuberculosis : primary infection , persistence , reactivation or active tuberculosis (19) .

Table (1) The phagocytic percentage mean after 15 minutes in control group and patients with primary and secondary TB.

	phagocytic percentage of study groups		
Values	Healthy Control	Patients with primary TB infection	Patients with secondary TB infection
Miniman	22	45	31
Maximum	58	63	56
Mean	40.25	61.94	45.09
SD	13.97	8.004	12.57
SE	3.13	1.94	3.39
N	20	17	11
Correlation		■	•

■ : Significant differences were observed when compared with control group .

• : No significant differences were observed when compared with control group .

SD:Stander diveation

SE:Stander error

Table (2) The phagocytic percentage mean after 30 minutes in control group and patients with primary and secondary TB.

	phagocytic percentage of study groups		
Values	Healthy Control	Patients with primary TB infection	Patients with secondary TB infection
Miniman	26	50	32
Maximum	61	70	68
Mean	43.75	64.06	43.27
SD	14.1	9.01	12.14
SE	3.15	2.19	3.66
N	20	17	11
Correlation		■	•

■ : Significant differences were observed when compared with control group .

• : No significant differences were observed when compared with control group .

SD:Stander diveation

SE:Stander error

Table (3) The phagocytic percentage mean after 45 minutes in control group and patients with primary and secondary TB.

	phagocytic percentage of study groups		
Values	Healthy Control	Patients with primary TB infection	Patients with secondary TB infection
Miniman	32	57	30
Maximum	63	76	70
Mean	42.95	66.24	45.36
SD	9.41	6.19	14.07
SE	2.1	1.5	4.24
N	20	17	11
Correlation		■	•

■ : Significant differences were observed when compared with control group .

• : No significant differences were observed when compared with control group .

SD:Stander diveation

SE:Stander error

Table (4) The phagocytic percentage mean after 60 minutes in control group and patients with primary and secondary TB.

	phagocytic percentage of study groups		
Values	Healthy Control	Patients with primary TB infection	Patients with secondary TB infection
Miniman	24	58	29
Maximum	65	81	68
Mean	47.75	68.53	41.73
SD	14.04	6.02	12.28
SE	3.14	1.46	3.7
N	20	17	11
Correlation		■	•

■ : Significant differences were observed when compared with control group .

• : No significant differences were observed when compared with control group .

SD:Stander diveation

SE:Stander error

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