Levels of Antibodies against Cytomegalovirus and Chlamydia Pneumoniae in Patients with E.P.H-Gestosis

Thesis

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By

Hoseiny Ahmed Hoseiny Kobtan
M.B., B.CH. (1997).
Tanta University

Supervised By

Prof. Mohamed Ashraf Mohamed Farouk Kortam
Professor of Obstetrics and Gynecology
Ain Shams University

Dr.Hesham Mahmoud Mohamed Harb
Assistant Professor of Obstetrics and Gynecology
Ain Shams University

Dr. Samia Abdou Girgis
Lecturer of Clinical and Chemical Pathology
Ain Shams University

Faculty of Medicine
Ain Shams University
Cairo
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مستويات الأجسام المضادة لفيروس السيتوميجالو والجرثومه المندثرة الرئوية في السيدات المصابات بمرض مقدمة الارتعاج

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الطبيب / حسيني احمد حسيني قبطان
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كلية الطب– جامعة طنطا

تحت إشراف

الأستاذ الدكتور/ محمد اشرف محمد فاروق قرطام
أستاذ أمراض النساء والتوليد
كلية الطب– جامعة عين شمس

الاستاذ الدكتور/ هشام محمود محمد حرب
أستاذ مساعد أمراض النساء والتوليد
كلية الطب– جامعة عين شمس

الدكتورة / سامية عبدة جرجس
مدرس الباثولوجية الإكلينيكية و الكيميائية
كلية الطب – جامعة عين شمس

كلية الطب
جامعة عين شمس – القاهرة
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Hoseiny Ahmed Hoseiny Kobtan

List of abbreviations

ACIF…… anticomplement immunoflurescent test
ACS…….. acute coronary syndrome
ANP……. atrial natriuretic peptide.
ATIII…….. anti-thrombin III.
C.Pneumonia Chlamydia pneumonia.
CAD…….. coronary artery disease
CFT……complement fixation test.
Chsp-60…… chlamydial heat shock protein-60
CMI……cell mediated immunity.
CMV……. cytomegalovirus
CPE……Cytopathic Effects
CRP…….. C-reactive protein
DFA……... direct fluorescent antibodies.
E.P.H……..... edema,proteinuria,hypertension.
EB……. elementary body.
EDHF……... endothelial-derived hyperpolarizing factor
EIA…… enzyme immuno-assay.
ELISA…… enzyme linked immunosorbent assay.
FA…… fluorescent antibody
HCG…… human chorionic gonadotrophin.
HCMV……... human cytomegalovirus
HDL……. high density lipoprotein
HSV……. herpes simplex virus.
HZV……. herpes zoster virus.
ICAM-1……. intercellular cell adhesion molecule-1
ICC……... immune cytochemical
IHA……. indirect hemagglutination test
IIF…… indirect immunoflurescence
IL…….....interleukin
IUGR……... intrauterine growth retardation.
IUK…… inactive urinary kallikrein.
LAT…….. latex agglutination test
LGV……lymphogranuloma venerium.
LHL…….. low density lipoprotein
LPS…… lipopolysaccharide.
MABs……..monoclonal antibodies.
MAC-5……monolayer culture-5.
MAP……. mean arterial pressure
MCMV…….. mouse cytomegalovirus
MCP……monocyte chemo attract protein.
MDV……... Maek's disease virus.

List of abbreviations (Cont.)

MI…….. myocardial infarction.
MIF…… micro immuno fluorescence.
MOMP…….. major outer membrane protein.
NO…….. nitric oxide.
NOS……. nitric oxide synthase
NT…… neutralization test
PAI-1…….. plasminogen activator inhibitor-1.
$PBMC_S$……. peripheral blood mononuclear cells
PCR…… polymerase chain reaction.
PDGF…… platelet-derived growth factor
PSTBM…… placental syncytiotrophoblast microvillous membrane.
RB……. reticulate body.
RCMV…….rate cytomegalovirus.
ROS…… reactive oxygen species.
SMC_S……. smooth muscle cells
TAT…… thrombin-antithrombin III complex.
TIA……. transient ischemic attack.
TN F-α…… tumour necrosis factor-α.
TVS…… transplant vascular sclerosis.
TWAR…….(TW…Taiwan)--AR….(Acute respiratory disease).
TXA_2…… thromboxane A_2
VCAM-1…..…. vascular cell adhesion molecule-1.
VEGF……... vascular endothelial growth factor.

مقـدمـــة :

يعتبر مرض مقدمة الارتعاج من أهم أسباب ارتفاع معدلات الإصابة والوفيات للأمهات والأطفال ويحدث هذا المرض نتيجة للاختلال في التوازن بين احتياجات الجنين والإمداد الدموي عبر المشيمة ويحدث أيضا هذا الاختلال في حالات تأخر نمو هذا الجنين بدون حدوث ارتفاع في ضغط الدم .

إن نقص الإمداد الدموي عبر المشيمة هو السبب الجذري لحدوث مرض مقدمة الارتعاج ويحدث هذا النقص نتيجة لحدوث ترسبات دهنية في الشرايين الرحمية تماماً كما يحدث في حالات مرض تصلب الشرايين .

هناك دلائل علي أن العدوى المزمنة بفيروس السيتوميجالو والجرثومه المندثره الرئويه يعتبران من العوامل المساعدة علي حدوث الترسبات الدهنية بالشرايين حيث تم العثور عليهما في الترسبات الدهنية في بطانة الشرايين .

كما يفترض أن فيروس السيتوميجالو والجرثومه المندثره الرئويه قد يساهم في حدوث تصلب الشرايين وذلك من خلال جذب خلايا الدم البيضاء (المونوسيت) لمناطق الاصابة بجدر الشرايين حيث تتكون الخلايا الرغوية ومن ثم تحدث الترسبات الدهنية ثم الجلطة بهذه الشرايين .

إن العدوى بفيروس السيتوميجالو والجرثومه المندثره الرئويه تعتبرعامل هام في حدوث مرض مقدمة الارتعاج وذلك من خلال زيادة الترسبات الدهنية في بطانة الشرايين

الرحمية والمشيمية وأيضا من خلال تقوية الاستجابة الالتهابية لجميع أعضاء جسم الأم الحامل .

إن العدوى السابقة أو الحالية أو المتكررة بالجرثومه المندثره الرئويه قد تساهم في حدوث تغيرات غير طبيعية في وظائف الأوعية الدموية وحدوث ترسبات دهنية بالشرايين المشيمية وبالتالي نقص الإمداد الدموي عبر المشيمة وهذا يمثل المرحلة الأولى في حدوث مرض مقدمة الارتعاج كما أن العدوى المزمنة بالجرثومه المندثره الرئويه قد تساهم أيضا في حدوث ثاني مرحلة من مرض مقدمة الارتعاج وذلك من خلال حدوث خلل في وظائف الخلايا المبطنة لجدار الأوعية الدموية .

CHLAMYDIAE

Historical aspect :-

Gordon and Quan (1965) separated Chlamydial isolates into two groups (A and B) on the basis of morphological and chemical characteristics of the organism alone . These workers found that Chlamydiae of subgroup (A) which are associated with trachoma, inclusion conjunctivitis, lymphogranuloma venereum and cytoplasmic inclusions containing glycogen stainable with iodine solution.

Lin and Moulder , (1966) confirmed these observations and detected that multiplication of Chlamydia agents of subgroup (A) was inhibited by sodium sulfadiazine. Other Chlamydial strains which originated from different avian mammalian hosts did not possess these properties and thus were placed in subgroup (B).

Page (1968) , proposed to recognize two species in the genus Chlamydia , Chlamydia trachomatis and Chlamydia psittaci .

Classification:-

Organisms were classified in order Chlamydiales, with one family Chlamydiaceae, one genus Chlamydia and three species C. trachomatis, C. psittaci and C. pneumoniae

Morphological characteristics :-

All members of the genus Chlamydia share a unique developmental cycle in which two distinct forms occur. These two forms are especially adapted for extracellular survival and transfer from cell to cell for intracellular growth ***(Jones., 1997)*** .

A) The Elementary body (EB) :-

The extracellular form, generally referred to as an elementary body, was a spherical bacterium 0.25 to 0.30 u in diameter. It was surrounded by a rigid trilaminar cell envelope similar in composition to those of other gram negative bacteria, except that the cell wall did not exhibit characteristic endotoxic properties. The inner side of the envelope was composed of a continuous layer of small snbunits structures in a hexagonal array and these subunits apparently serve to maintain envelope rigidity. The cytoplasm of the elementary body was surrounded by plasma membrane (***Jawctz et al. , 1992***).

The DNA and ribosomes were condensed in the center of the organisms, and in electron micrographs of shadow-cast preparation these assume a "derby hot" or fried egg appearance. Thin section electron microscopy shows a similar central condensation (***Chi et al., 1987***).

B) The Reticulate Body (R .B):-

The intracellular form of Chlamydia referred to as the reticulate body was much larger than extracellular form (about 100 mm in diameter) and differs from it in many respects. It was surrounded by a trilaminar envelope that was very fragile and flexible so that pleomorphism results (***Moulder , 1991***) .

The subunit layer found on the inside of the elementary body envelope was missing, and no sulfur containing amino acids had been detected in the outer cell wall. Ribosomes and other cytoplasmic constituents were distributed homogeneously throughout the cytoplasm (***Moulder, 1991***) .

The difference between elementary body (EB) and reticulate body (R.B) is presented in table (4) .

Staining properties :

Chlamydiae are gram negative bacteria. They stain blue by Castaneda's method and red by Macchiavello and Giemenez stain ; the latter two stains or Giemsa stain are most often used to demonstrate the organisms in impression of the chick embryo and yolk sac. Whereas, Giemsa is the stain of choice for inclusions in cell cultures (***Cartwright, 1988***). Giemsa stained preparations, when examined by light microscopy show the difference between the small reddish-purple EB and the larger basophilic RB. By dark field microscopy, the Giemsa stained preparations show the EBs as brilliant yellow particles (***Banie, 1990***) Not only that, but also staining reactions with Giemsa suggest that the EB contain DNA and the RB a preponderance of RNA. This is continued by acridine orange staining with which these particles stain respectively yellow green and orange (***Closs et al., 1991***) .

Chlamydia Trachomatis inclusions appear as well defined rigid structures contained within a limiting membrane, Chlamydia psittaci inclusions tend to be so well defined as those of Chlamydia trachomatis and are generally larger and more irregular in appearance. The iodine method is a simple and rapid way of demonstrating inclusion in cell

cultures , it is of no value for impression smears of yolk sac in which inclusions are disrupted ***(Evand and Wooblant 1983).***

Table(2) Difference between elementary body and reticulate body .

Characteristics	Elementary Bodies	Reticulate bodies
1- Size	0.2-0.3 mM	1.0mM
2- Mophology	Electron dense rigid core	Pleomorphic gram –ve coccus
3- Infectivity to host cell	Infectious (responsible for cell to cell and host to host transmission)	Non infectious .
4- DNA : RNA	1 : 1 .	3 : 1
5- Metabolic Activity	Inactive	Active
6- Stability	Stable	Non stable outside host cell
7- Haemagglutiation	Present in some strains	Absent
8- Envelop subunit	Present in some strains	Absent

Quoted from Moulder (1991)

Life cycle:-

Chlamydiae have a unique life cycle that distinguishes them from other organisms. The life cycle involves an infections but metabolically inactive elementary body (EB), and a reticulate body (RB) that actively synthesizes DNA and other molecules. Attachment of the (EB) to the host cell is receptor specific, thus explaining the predilection of Chlamydia trachomatis for columnar cells. Penetration of the host cell is by active enhanced phagocytosis induced by the parasite. After phagocytosis, the organism remain within the phagocytic sac, and somehow prevent lysosomal fusion by the host cell. The organism's ability to induce phagocytosis and to prevent lysosomal fusion are its main virulence factors (***Jones , 1997***).

After phagocytosis, the (EB) transforms into (RB), which then uses host cell mitochondria to power the synthesis of DNA, RNA and glycogen. It is the glycogen accumulation that accounts for the (RB) being stainable with iodine (***Grayston, 1992a***) .

The (RB) being dividing by binary fission from 8-24 hours after phagocytosis. This is the stage of greatest metabolic activity. Then the (RB) recognize into (EB). Beyond 18-24 hours, the (EB) increase in number and appear to predominate in the inclusion bodies. This entire cycle takes place within the phagosome (phagocytic sac). Which

obviously undergoes a large increase in size, then ruptures after 48-72 hours later to release the infectious elementary bodies (***Cartwright, 1988***).

Moulder, (1991) suggested that the phagosomal membrane enveloping (EB) carries a marker that discourages fusion with lysosomes. ***Mandell et al (1997)*** discovered that on the initiation of the life cycle when an EB attaches to a epithelial cell it uses heparin sulfate as a bridge between receptors on its surface and that of the target cell. Attachment is receptor specific thus explaining the predilection of C. trachomatis for columnar cells .

Antigenic composition :-

Chlamydiae are highly complex micro-organisms which possess a number of different antigens of genus, species and subspecies (serotype) specificily (***Shachter and Caldwell., 1990***) .

A-Genus specific Antigens :-

All Chlamydiae share a common complement fixing genus specific antigen. This genus antigen is polysaccharide in nature, heat stable, peroxidase sensitive and can be extracted from infected tissues with ether or detergent as deoxycholate (***Cartwright.,1988***). It is contained in the cell wall and is antigenically similar to the lipopolysaccharidc of Acinelobacter and Remutants of Salmonella species (***Nurminen et al., 1993) .***

B-Species specific antigens :-

These antigens are probably membrane associated and can be demonstrated by indirect haemagglutination and immuno-electrophoresis adsorption with monospecific antibody. Antibody to species specific antigen can be used to differentiate Chlamydia psittaci from Chlamydia trachomatis ***(Schachter and Caldwell, 1990).***

C- Type specific antigen :-

The subspecies and or serotype specific antigens are common only to selected strains within Chlamydial species. These antigens have been the basis for a variety of serological tests used for classification of Chlamydia trachomatis serotypes. They are trypsin and heat labile proteins present at the surface of the elementary body. Antibodies to these cell wall type specific antigens seem to prevent infection of host cell, but have little effect on Chlamydiae that have already established an intracellular existence ***(Schachter and Onossman . , 1991)***.

CHLAMYDIA PNEUMONIAE

Historical aspect :-

TWAR is a strain designation which was derived from the laboratory codes of the first two isolates TW-183 and AR-39. TW-183 was isolated in 1965 from the conjunctiva of a control child in a trachoma vaccine study in Taiwan. It was the 183rd chick embryo egg yolk sac isolate of a Chlamydia organism from a series of studies of trachoma. The strain was untypable as trachoma strain by die mouse toxicity prevention test (***Grayston et al., 1986).***

Several years later when cell culture of Chlamydia was developed, it was found that the inclusions TW-183 found in cell culture were round and dense unlike Chlamydia trachomatis strains, it was therefore assumed to be Chlamydia psittaci strain. AR-39 came from a throat swab of university of Washington student with pharyngitis in 1983 (***Ekinan et al., 1993) .***

Serosurveys indicate that these " TWAR" strains have a world wide distribution (TW) from Taiwan, reflecting geographic locate of the first isolate, (AR) for acute respiratory disease association (***Scliocliter, 1990***). The species name for the TWAR organism is officially Chlamydia pneumoniae (***Grayston et al., 1993***).

Transmission-:

Chlamydia pneumoniae (TWAR) is emerging as an important cause of respiratory disease in humans it has been implicated as a cause of bronchitis, pneumonia, sinusitis and pharyngitis ***(Grayston et al., 1990) .***

Although the disease is presumed to be spread via the respiratory route from person to person the actual mechanism of transmission is not known ***(Thom et al., 1992).***

Three modes of transmission of respiratory pathogens are theoretically possible as small aerosol generated by talking , coughing or sneezing can spread for distance of 71.8 cm. Direct inoculation by droplet or large particles requires close person to person contact and inoculation after touching contaminated surfaces requires that the organism remain viable in the enviroment and on skin for sufficient time to allow transmission. Spread of infection by the latter two modes tend to be slower ***(Falsey and Walsh, 1993)*** .

Epidemiology-:

Seroepidemiological studies of existing serum banks of respiratory infections. It is clear that the organism is not new, but has been causing pneumonia and other respiratory syndromes for many years (***Grayston, 1997***). Population prevalence antibody studies have shown that at least 50% of adults have antibody (IgG serum fraction titer 16 to 256) in many countries around the world (***Roblein et al , 1992***) .

Age-specific rates of antibody in the United States and Northern Europe show that antibody is uncommon under the age five years. The rate increases rapidly from 5 to 20 years of age and then increases slowly into old age. Men over 60 have a prevalence of 70%. Rates are higher in men than women after the age of 20 years ***(Campbell et al., 1993).***

Pathogenicity :

After inhalation, Chlamydia pneumoniae enters the blood stream from the respiratory tract and travels to the reticuloendothelial cells of the liver and spleen. The pathological findings in the lungs are congested alveolar capillaries and alveolar spaces containing protein aceous liquid,alveolar lining cells, red blood cells, fibrin and white blood cells. The leukocytes including lymphocytes, macrophage , monocytes and occasionally, neutrophiles. Alveolar septate may become necrotic from edema and infiltration with lymphocytes and mononuclear cells ***(Jones, 1997) .***

Small bronchioles are either unaffected or rare sites of inflammation. Within macrophages and alveolar lining cells the intracytoplasmic inclusion bodies may be visible with the Giemsa stain, pleural involvement is uncommon, but may take the form of mild fibrinous pleuritis or pleural petechiae ***(Roblin et al., 1992) .***

The spleen is usually enlarged and congested, with lymphocytic infiltration of the pulp and increased numbers of phagocytes that sometimes contained ingested erythrocytes.The liver is often enlarged, showing histologic changes of swelling, vacuolization. and increased phagocytic activity of the Kupffer cells. Occasionally, myofibrillar damage, interstitial edema and inflammation with lymphocytes, plasma cells, and neutrophiles are found in the heart (***Hirschmann., 1989***) .

Clinical features:

The incubation period of infection due to C. pneumoniae is several weeks, which is longer than that for many other respiratory pathogens ***(Marchers et al., 1994).***

I. Respiratory Infections:

Pneumonia and bronchitis are the most frequently recognized illnesses associated with C. pneumoniae, although asymptomatic infection or unrecognized, mildly symptomatic illnesses are the most common result of infection. In a series of studies, 10 % of cases of pneumonia and approximately 5 % of bronchitis and sinusitis cases in adults have been attributed to the organism ***(Grayston., 1992).***

II. Severe Systemic Infecticn:

Severe systemic infection with C. pneumoniae, while uncommon, do occur. there are several severe adult cases with serological evidence of C. pneumonia infection only. It is also identified in autopsy tissue by PCR , suggesting that the organism played at least a part in the infectious process prior to death. A commercial laboratory found very high TWAR MIF IgG serum antibody, which lead to further studies that resulted in a PCR demonstration of C. pneumoniae in stored lymph node and liver biopsy specimens ***(Kuo et al., 1995).***

III. Other Syndromes:

TWAR has also been associated with other acute illnesses. It has been isolated from patients with purulent sinusitis ***(Hashigucci et al., 1992)*** and otitis media with effusion ***(Ogawa et al., 1992).*** Primary pharyngitis due to C. pneumoniae has been reported; however, the frequency of this infection is unclear, while C. pneumoniae infection has been reported in as high as 8 % of adults with pharyngitis in Finland ***(Huovinen et al., 1989)***, it appears to be uncommon (less than 2 % of cases) in studies of young adults in the United States ***(Hammerschlag et al., 1992; Thorn et al., 1990).*** Other reported clinical syndromes include endocarditis ***(Marrie et al.,1990)*** and lumbosacral meningoradiculitis ***(michel et al.1992).***

Several chronic diseases have also been presumptively associated with C. pneumoniae infection. Patients with C. pneumoniae respiratory infection have been shown to be more likely to develop asthmatic bronchitis following their respiratory illness, suggesting that TWAR may be a factor in the development of asthma or asthma exacerbation . C. pneumoniae has been associated with sarcoidosis by serologic studies ***(Black et al. 1992; Gror, hagen-Riska et al., 1988)*** and with erythema nodosum (***Erntell et al., 1989;Sundelof et al., 1993).***

A case of Guillain-Barre Syndrome following infection with C. pneumoniae has been reported ***(HaidI et al., 1992).*** C. pneumoniae has also been implicated in reactive arthritis or Reiter's syndrome ***(Braun et al.1994).***