Antibodies to Epstein – Barr – virus(kissing Disease) in Thalassemic Patients

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Summary

Background :To detect and study prevalence of Epstein Barr – virus in human serum of thalassemic patients in Mosul –Iraq.

Patients and Methods

The presence of IgM – antibodies has been examined in a group of 104 (63 males and 41 females) consecutive thalassemic patient who received blood transfusion in Mosul .

Results

Overall 18.3% were found to be anti – EBV positive and 81.7% were negative and Only 6.0 (20.7%) from those positive were also have anti lgM for Cytomegalovirus CMV.

Conclusion (s):

Epstein – Barr –virus infection was diagnosed by serological screening in 19(18.3%) thalasemic patients in Mosul using ELISA.

Key words:

Epstein – Barr –virus, thalassemic, IgM, Infectious Mononucleosis.

الأجسام المضادة لفيروس ابشتاين- بار(مرض التقبيل) في مرضى الثلاسيميا

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ألخلاصة

الغاية :-

للتحري ودراسة انتشار الأجسام المضادة لفيروس ابشتاين- بار في مصول مرضى الثلاسيميا في الموصل- العراق.

المرضى وطرائق العمل:-

تم التحري عن وجود الكلوبيولينات نوع أم في مجموعة من 104(63ذكرو41 أنثى) في مرضى يعانون الثلاسيميا والذين يستلمون الدم في الموصل.

النتائج:-

اعطى18.3 % منهم أجساما مضادة ضد فيروس ابشتاين- بار ولم تلاحظ في81.7% منهم .وأعطى فقط من الذين كانوا يمتلكون هذه الأجسام أيضا أجسام مضادة لفيروس التشوهات الخلقية CMV.

الاستنتاج:-

شخصت الاصابه بفيروس ابشتاين-بار بالتحري مصليا في19(18.3%) من مرضى الثلاسيميا.

Introduction

Herpes virus family contains several of the most important human pathogens. The outstanding properly of Herpes viruses is their ability to establish life long persistent infection and to undergo periodic reactivation in immunosuppressed patients. There are nearly 100 viruses of this group that infect many different animal species (1, 2). The useful division of the

Herpesviridae into subfamilies is based on biological properties it is include Alpha herpes viruses Betaherpes viruses and Gameherpes viruses exemplified by Epstein – Barr- Virus (EBV). From genus lymphocryptovirus and species Human herpes virus 4 (HHV-4) which infect and become latent in lymphoid cells especially in epithelial cells of oropharyno and paratoid glands and uterine cervix .(1,3,4) .It cause infectious mononucleosis and is the cause of human lymphoproliferative disorders .EBV initiats infection of B cells by binding to the viral receptors C3d component of complement (CR2 or CD21) and directly enters latent state in lymphocytes but the efficiency of B cell immortalization by EBV is quite high which express differentiated functions , such as secretion of Igs (5,6) . Thalassemia is an inherited autosomal recessive blood disease . the genetic defect results reduced of synthesis of one of globins chain that make up hemoglobin and cause the formation of abnormal molecules which cause anemia the characteristics of thalassemia (7) .

Hemoglobin is mainly made up of 2 kind of proteins α & B. Individuals with thalassemia do not produce enough of one (or occasionally both of these proteins so their RBCs may be abnormal & unable to carry enough oxygen (8)In α - thalassemia , production of α -globulin chain is affected , which in B-thalassemia production of B- globulin chain is affected (7).Common serologic procedures for detection of EBV antibodies include ELISA tests , immunoblot assay using EBV – positive lymphoid cell(9) .Early in acute disease , transient rise in IgM .

Antibodies to viral capsid antigen occurs, replaced within weeks by IgG Antibodies to this Ag which persist for life. In the course of infection mononucleosis, most patients develop transient heterophil antibodies that agglutinate sheep cells (10, 11) the presence of antibody of IgM type to viral capsid antigen is indicative of current infection. Antibody of IgG type is a marker of post infection and indicate immunity though such antibodies are often found in patients with Burkitt lymphoma or nasopharyngal Carcinoma so detection of rise in anti-EBV antibody would suggest primary infection (1,2,8) Not all persons develop antibody to EBV .

EBV common in all parts of the world with over 95% of adults between 35-40 years of age have been infected . It is transmitted by primarily contact with oropharyngeal secretions. In developing areas, infection occurs early in life, more that 90% of children are infected by age 6. These infections in early childhood usually occur without any recognizable disease (12). The apparent infections result in permanent immunity to infectious mononucleosis. In industrialized nations more than 50% of EBV infections are delayed until late adolescence and young adulthood. In almost half of cases the infection is manifested by infectious mononucleosis. There are 100.000 cases of this disease in United States (10, 11, 13). Several studies have connected lytic activity and reactivation with rejection episodes in transplant 3 recipient posttransplant lymphoproliferative disorders and in patients with multiple sclerosis (12) Until now , EBV reactivation has been diagnosed only by mean of serology (11,12). Serology reflects reactivation only retrospectively and does not represent the actual event. Especially under immunosuppression , serologic response may be delayed , but as immediate diagnosis of EBV reactivation is of importance to prevent or overt sever complications .

we wanted to investigate diagnoses of EBV reactivation by ELISA in thalassemic patients .There is no vaccine a available to EBV Acyclovir reduce EBV shedding from or pharynx during period of drug administration and has no effect on symptom of mononucleosis & is of no proved benefit in treatment of EBV associated lymphomas in (9) immunocompromised patients .

Material & Method

EBV Serology was determined using Enzyme linked immunosorbent Assay (ELISA) which is intended for detection of IgM class antibodies to EBV capsid antigen in human serum from the ELISA employed EBV purified capsid Ag (EBVCA) Human Gesellschaft for Biochemic and Diagnostic mbH Max- Planck –Ring 21-65-205 wiesbaden – Germany (13,14)& also for CMV (IgM) from the same source . The same procedure was performaed for detection of CMV (IgM) ELISA in seropositive EBV serum only.The major Ag in the VCA complex are P150 (Bc LFI) P18 (BFRF3) P23 (BLRF2) and gp 110 knows as gp125 (BALF4)

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Method

- The cell culture derived EBV capsid antigen (EBV Ag) which is bound via specific monoclonal antibodies is coated on microtiter wells as a solid phase.
- Dilute patient serum is added to the wells and corresponding specific antibodies (EBV – IgM Ab) if present, binds to the antigen during incubation at 17-25 °C for 30 minutes.
- After washing the wells to remove unbound component (anti-human IgM antibodies, peroxidase conjugated).is added and incubated at 17-25°C for 30 minutes.
- Unbound conjugate is removed by a subsequent washing step.
- A solution of TMB/ substrate reagent is then added to the microtiter wells.
- The enzyme conjugate catalytic reaction is stopped at specific time.
- The intensity of the color generated is directly proportional to the EBV – IgM- Ab concentration the sample.
- The absorbance of control and specimen is determined by using microplate readers at 430 nm within 30 m
- The result for patient samples is obtained by comparison with a cut – off value.

The our study was carred out from sept-2009- feb. 2010 in Iraq. 104 thalassemic patient attending the thalassemic center in Mosul .
Statistical analysis was performed using the fisher free man – Halten test & chi- square take to compare difference between populations.
Seropositivily was defined for EBVCA as value of > 0.444 relative units and was defend for EBVCA IgM a ratio (extinction sample / extinction calibrator) of more > 0.386.

Results & Discussion

Over all (104) patients (63 males & 41 female) 18.3% were anti – EBVCA positive.
Table (1) shows anti-EBV seropositivty according to Age & sex.

Table -1- percentage& Number of anti– EBVCA (IgM) according to Age & sex .

Sex	Age group	N (%) -Ve	N (%) +Ve	Total
Males	1-10 11-20 21-35	19(36.2) 24(46.2) 9(17.3)	2(18.2) 8(72.7) 1(9.1)	21(33.37) 32(50.87) 10(15.9)
Total		52(100%)	11(100%)	63(100%)
Females	1-10 11-20 21-35	14(42.4) 17(51.5) 2(6.1)	3(37.5) 5(62.5)	17(41.5) 22(53.7) 2(4.9)
Total		33(100)	8(100)	41(100)

No difference was registered over P> 0.187. In the present study (11) male & (8) females were positive for EBVCA lgM .
These subjects have reactivated or persistently active EBV infection. In present study(52) male & (33) femal indicates that healthy subjects. Serologic responses are more suggestive of no viral replication although serologic investigation did not determine exact reactivation time point: on the contrary.
Screening of anti- CMV (IgM) by ELISA also for only the positive serum of EBVCA indicate only 6(20.7%) were found positive as shown in table -2-.
The reason for this remains uncertain but might be due to a lower of sensitivity of the serologic assay used & available.

Table -2- Percentage of presence of anti – CMV (IgM) within anti-EBV (IgM) in patient's serum.

	Anti-CMV (IgM)		
Anti-EBV	-Ve N(%)	+Ve N(%)	Total
-Ve N(%)	62 (82.7)	23 (79.3)	85 (81.7)
+Ve N(%)	13 (17.3)	6 (20.7)	19 (18.3)
Total	75 (100)	29 (100)	104 (100)

The data reported confirm that EBV& CMV infection is two of major clinical problems for those patients who required transfusion therapy . So before transfusion therapy the EBV & CMV Screening become available in these patients.

The probability of being seropositive is proportionally related to the number of transfusion – However. The study showed that 85 from 104 patients haven't anti – EBV (IgM) , the fact that suggest that it can be transmitted not only via blood & injection but also by contaminated , amniotic fluid saliva , throat swabs or others ,thus infection of EBV & CMV may be possibly spread though routes other than over all parental .The presence of IgM antibodies is used as a marker of recent EBV infection . It's usually position at time of presentation with infections . Mononucleosis and persists for only few weeks , because most EBV infection occur in child-hood.

It is difficult to determine if the antibody patterns are similar in in apparent versus symptom ties disease . Som studies have shown that testing for antibodies to P18 may be insensitive in early of the invectives mononucleosis (OS1) of less suitable for user (11,14) .

There is no vaccine available to EBV (5) . Acyclovir reduce EBV shedding from oropharynx during period of drugs administration and has no effect on symptoms of mononucleosis and is of no proved benefit in treatme (8,16) .

In conclusion this study has demonstrated that EBV is presence in thalasemic patients & the results indica no significance correlation between EBV & CMV.

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