Brief Report

Comparison of serological and molecular test for diagnosis of infectious mononucleosis

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Abstract

Background: Epstein-Bar virus (EBV) is the main etiology of infectious mononucleosis (IM) syndrome that is characterized by fever, sore throat, and lymph adenopathy. Since, this virus could be associated with a number of malignancies, some hematologic disorders, and chronic fatigue syndrome, identification of IM is very important.

The aim of study was to evaluate the specificity, as well as sensitivity of the two different methods that is, serology versus molecular diagnosis that are currently used for diagnosis of IM.

Materials and Methods: In this study, during a period of 3.5 years, 100 suspected patients as case group and 100 healthy individuals as a control group were studied. Fifty samples in each group were tested by polymerase chain reaction (PCR) and all the samples including case group and control group were carried out by enzyme-linked immunosorbent assay (ELISA).

Results: In 76% of patients and in 20% of the healthy individuals, samples were detected EBV DNA by PCR. On the other hand, 68.5% of the samples belong to the case group and 46% in the control group showed positivity by ELISA.

Conclusion: By comparing the two methods, since PCR is very expensive and time consuming, and the percentages of difference ranges are narrow, ELISA could be applied as a first, easiest, and preliminary diagnostic test for IM. In addition, this test could be applied in various phases of the disease with a higher sensitivity comparing to PCR.

Although PCR is routinely used for diagnosis of various infectious agents, it is considered as an expensive test and merely could be used after 1-2 weeks from the onset of the illness.

Key Words*:* Epstein Bar virus, enzyme-linked immunosorbent assay, molecular, mononucleosis

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INTRODUCTION

Infectious mononucleosis (IM) is a viral disease, which is presented by fever, sore throat, lymphadenopathy, splenomegaly and hepatic inflammation. The etiology of IM is Epstein–Barr

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virus (EBV) which belongs to the herpes virus family.[1]

Due to the integration of DNA of EBV in B lymphocytes and reticuloendothelial system, nasopharyngeal carcinoma,Burkit's lymphoma, and gastric cancer are late complications of this infection.[2,3] Leukopenia, thrombocytopenia, hemolytic anemia, pancytopenia and hepatitis are also early complications of this diseases.[4,5]

Every organ could be involved in IM, but the main problems are obstruction of the airway due to hypertrophy and hyperplasia of tonsillar tissues, hepatitis, obstructive jaundice especially in elderly, myocarditis, thrombocytopenia, autoimmune hemolytic anemia and splenic rupture.[5] IM might cause chronic fatigue syndrome.[6]

DNA of the virus is detectable in blood and B cells in the acute phase, and its peak is about the 2nd week from the onset of the disease.But however, after a few weeks to few months, it disappears, and antibody (Ab) appears in the serum.[7]

Diagnosis of IM is based on clinical, hematological and serological data. From clinical aspect, fever, sore throat, and lymphadenopathy are the triad of disease. Thrombocytopenia, hemolytic anemia, and lymphocytosis are prominent hematologic disorders. Almost signs and symptoms of IM are due to the proliferation and over activity of T lymphocytes in response to the virus.[1]

Appearance of heterophil Ab (mono test) is seen in 90% of the adult patients in classical cases about 4 weeks after the beginning of IM (but in children the sensitivity of the test is decreased to 33%).[8]

Specific IgM and IgG (Ab) appear against viral capsid antigen (VCA), Epstein-Bar nuclear antigen (EBNA) and early antigen (EA) depends on different stages of the disease.[9] In prodromal phase, Ab is negative and in the acute phase, IgM against VCA appears and then switches to IgG.[9]

In the acute phase, there is no IgG against EBNA. In this phase, some patients present EA Ab. In convalescent phase IgG EBNA appears, and IgG VCA exists. In healthy individuals with past infection, IgG VCA and IgG EBNA are present in 90%.[9]

Relapses of virus activity in past infected healthy individuals are possible.[5, 10]

Diagram of appearance of Ab against viral antigens is based on chronological order.[7] The presence of IgM against VCA shows acute phase of the disease, whereas appearance of IgG against EBNA indicates the convalescence or chronic phase of the disease.[11,12]

Diagnosis of IM is done through serological and molecular assay. Serological tests, which are routinely done for diagnosis of the EBV, are IgM and IgG VCA, IgG EBNA, EA.[12]

Polymerase chain reaction (PCR) as a diagnostic test is offered by Telenti and colleagues in 1990 for detection of viral DNA.[13]

Balfour and colleagues categorized stages of the disease by viral load in PCR diagnosis.[12] In their 697 sample, 25 specimens were in primary infection and based on his study, adult patients could clear blood from virus in early stage but it survives in pharynx. The PCR has been used to detect EBV-DNA in cell free serum or plasma samples of patients with primary and persistent EBV infection.[14]

The aim of study was to evaluate the specificity, as well as sensitivity of the two different methods that is serology versus molecular diagnosis that are currently used for diagnosis of IM.

MATERIALS AND METHODS

In a case control study from 1389 to 1391, 100 suspected patients diagnosed based on inclusion criteria such as fever, sore throat, and generalized lymph adenopathy and included in this study as case group. On the other hand, 100 healthy individuals were chosen from patients' families whose age and gender were the same. Our exclusion criteria were: LACK of cases' cooperation. Calculations related to sample size were carried out on the basis of previous studies in order to detect a difference in the percentage of patients who had 75% versus 45% prevalence, and in order to achieve a power level of 90%, with an alpha error of 1%, based on , $n=\frac{(Z_{1-\alpha/2}+Z_{1-\beta})^2\,[P_1\,(1-P_1)+P_2\,(1-P_2)]}{(P_1-P_2)^2}$ we needed 73 patients to assign them to each group.

For all the samples, enzyme- immunosorbent assay linked (ELISA) was done by using kits obtained from (EUROIMMUN, Medizinische, Labordiagnostika, Germany) on sera. PCR was done for 50 samples from case group and 50 from control group.

Polymerase chain reaction assay

Four micro liters of DNA elute was used for a first PCR amplification with a 10 pmol concentration of each sense and antisense primer (5'-AAG GAG GGT

GGT TTG GAA AG-3' and 5'-AAC AGA CAA TGG ACT CCC TTA G-3'), respectively, corresponding to the EBNA-1 gene of EBV to detect EBV DNA. The PCR mixture (25 µl) contained PCR buffer (10 mM Tris-HCl, 2.5 mM $MgCl_2$, 50 mM KCl, 0.1% gelation [pH =8.3], 10 mM deoxynucleoside triphosphate and 1 U of taq polymerase (Cinagen, IRAN). Samples were then subjected to cycles of amplification (5 min at 94^0C, 30 s at 94°C, 30 s at 56°C and 45 s at 72°C) in thermal cycler (Eppendrof, Germany) followed by extension at 72^0C for 5 min. The PCR mixture was used similar to that of the first PCR. In each experiment, a negative control composed of either sterile water instead of genomic or serum human DNA was tested, as well as a positive control EBV DNA.

Enzyme-linked immunosorbent assay

Patients' sera were assayed to determine VCA IgG, VCA IgM, and EBNA IgG using commercial ELISA kit (EUROIMMUN, Medizinische, Labordiagnostika, Germany) according to manufacturer's instructions. Results of the VCA IgM, VCA IgG, and EBNA-1 IgG Ab assays were clarified according to their index value as negative (<0.80), equivocal (0.80–1.1), or positive (>1.10).

Results were analyzed and compared in each group by Fisher's exact (SPSS 18.0). $P < 0.05$ was considered statistically significant.

RESULTS

By PCR, 38 (76%) cases of the case group were positive, and 12 (24%) were negative. In the control group, only 20% were positive, and 80% were negative [Table 1]. There was a significant difference between two groups by Fisher's exact test ($P = 0.0001$).

Detection of IgM and IgG against VCA and IgG against EBNA by ELISA test was done on total samples of case and control group. Cut of point titer <0.8= negative and >1.1 positive and between these titers borderline (±).

About 80% cases in the case group were positive for IgG VCA and 32% for IgM VCA. In the control group 100% of specimen was negative for IgM VCA and 73% positive for IgG VCA [Table 2]. Comparing results from case group to control group shows no significant difference between two groups ($P = 0.5589$).

For IgG EBNA 60% of case group and 30% of control group were positive [Table 3], with a significant difference between two groups ($P = 0.0002$), (Fisher's test).

Table 1: Results of PCR in case and control groups

Samples	Total number	Specimen	Results (%)	
			Positive	Negative
Case group	50	Blood	38 (76)	12 (24)
Control group	50	Blood	10 (20)	40 (80)

PCR: Polymerase chain reaction

DISCUSSION

Regarding PCR results, 38 samples in case group and 10 samples in the control group showed positivity by PCR. In a study done by She *et al*. in 2001,49 out of 70 patients and 3 out of 70 healthy control showed positivity by PCR, which is in accordance with our study,[15,16] which is approximately compatible with our study. The presence of EBV DNA in only 10 subjects of 50 healthy controls indicates that although most such individuals should be expected to be carrying EBV DNA in their lymphocytes, EBV DNA is not usually found in serum in the absence of active EBV disease, confirming previous reports.[13]

In the Okay *et al*., study in 2005 PCR was positive in 10 out of 15 cases,[15,17] which is compatible with our study.

The positivity obtained by ELISA for IgM and IgG against VCA and IgG against EBNA were 35%, 80% and 60% in case group, respectively. Whereas results gained in control group showed positivity in 6%, 76% and 25% cases, respectively. DNA of the virus was detected in 76% of patients and 20% in healthy individuals respectively. Since, IgM VCA is used as a main indicative marker for diagnosis of acute mononucleosis infection, comparing the results obtained by PCR (76%) and ELISA (35%) by Chi-square test shows significant differences ($P = 0.005$). Moreover, because the Ab is detectable in recurrent infection with this virus, therefore it is recommended that a test for EBNA be along with VCA IgG and/or IgM for the diagnosis of primary EBV infection to be done.

Table 2: ELISA test for IgM and IgG VCA in case and control groups

Antibody	Case group (%)			Control G (%)		
	Positive	Negative	Positive or negative	Positive	Negative	Positive or negative
IgM VCA	32	63	5	-	100	-
IgG VAC	80	15	5	73	27	-
Total	100			100		

VCA: Viral capsid antigen

Table 3: ELISA test for IgG EBNA in case and control groups

Antibody	Case group (%)			Control G (%)		
	Positive	Negative	Positive or negative	Positive	Negative	Positive or negative
IgG EBNA	60	35	5	30	70	-
Total	100			100		

EBNA: Epstein–Barr nuclear antigen

A reliable test for EBNA Abs can be used as a screening test since the presence of EBNA Abs excludes primary EBV infection. Abs against other EBV antigens must then be done only if EBNA Abs is absent.

Since, IgM VCA is used as a main indicative marker for diagnosis of acute mononucleosis infection, comparing the results obtained by PCR (76%) and ELISA (35%) by Chi-square test shows significant differences ($P = 0.005$).

CONCLUSION

Comparing the results obtained by PCR and ELISA, in this study shows that ELISA could be used as an appropriate, sensitive and specific test for diagnosis of EBV and management of IM. Due to following reasons, ELISA could be a useful test:
- Antibody against VCA, EBNA is detectable in every stage of disease for screening as well as diagnosis of IM (acute, convalescent, recent and past infection)
- Because of ngraisi of different types of Ab in various phases of the disease, ELISA is very useful for diagnosis of IM
- ELISA could be used as a differential test for diagnosis of patients with past infection with EBV and patients with sore throat with other infectious agents
- PCR is a time consuming and expensive test
- The availability of the PCR is not the same as ELISA.

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Conflicts of interest
There are no conflicts of interest.

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