Original Article

The role of Interferon-gamma(IFN-γ) in infectious mononucleosis like syndrome

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Summary

Background: *Infectious mononucleosis is caused by the ubiquitous Epstein-Barr virus .It is a common condition usually adolescents and young adults. Most cases are mild to moderate in severity with full recovery taking place over several weeks. More sever cases and unusual complications occasionally occur.*

Aims: *Determining the role of Th1and Th2 in Infectious mononucleosis –like syndrome by measuring IFN-gamma.*

Method: *Enzyme immunoassay for Quantitative Determination of human Interferon-gamma in serum.*

Results: *The result of serum levels of IFN-gamma in the control were (5.12±0.8)pg/ml significantly lower than that in patients with EBV infection (60.87±2.498)pg/ml. In patients with non EBV infection the levels of IFN-gamma were 55.850±0.825)pg/ml which were significantly lower than patients with EBV group ($p<0.001$).There was high significant difference between EBV, Non EBV and Healthy control groups .*

Conclusion: *High levels of IFN-gamma may be referred to a mixed TH1/TH2 response in infectious mononucleosis –like syndrome.*

Keywords:Epstein Barr virus,NonEpstein Barr virus ,IFN-gamma

Introduction:

Infectious mononucleosis-like syndrome (IMLS) is an acute infection characterized by high fever, sore throat and lymphadenopathy especially in the cervical lymph nods. It is mainly caused by Epstein-Barr virus (EBV), a gamma herpes virus that is believed to infect 90% of the world's population. It's most common presentation is a flue-like illness called Infectious mononucleosis, which usually resolves on it's own, but can also be caused by the Cytomegalovirus (CMV), Herpes simplex viruses (HSV)-1 and 2, Varicella- zoster virus (VZV) and Human herpes viruses (HHV) -6, 7 and 8. These viruses are members of one family Herpesviridae, all of them share properties including a genome of double-stranded linear DNA core surrounded by an icosahedra nucleocapsides symmetry, and a viral envelop (1, 2). They also share the biological properties of latency and reactivation, which cause recurrent infections in the host (2).

Toxoplasma gondii, Hepatitis A virus (HAV), Rubella, Human immunodeficiency virus (HIV), Adeno virus, *Corynebacterium diphtheriae*, Betahemolytic streptococci, and other agents are associated with IMLS as well (3). the surface of B cells and epithelial cells, is also the receptor for the C3d component of the complement system. It is expressed on B-cells and epithelial cells of the oropharynx and nasopharynx. Infection of the epithelial cells of the oro- and nasopharynx is permissive. The virus is sheded into the saliva and infects B lymphocytes in lymphatic tissue and blood (4).

Cytokines

Cytokines are low molecular proteins, it is a soluble mediators produced by a variety of cells. Their major functional activities are concerned with the regulation of the development and behavior of the immune effector cells (Benjamini et al.,2000)5.Cytokines serve as chemical messenger within immune system, although thy also communicate certain cells in other systems, including those of the nervous system. Cells regulated by a particular cytokine must express a receptor for that factor. Thus; cells are regulated by the quantity and type of cytokines to which they are exposed and by the expression up regulation and down regulation of cytokine receptors. Cytokines act in concert with one another to create synergistic effects that reinforce the other actions on a given cell. The interactions of multiple cytokines generated during a typical immune response are referred to a cytokine cascade (Hunter and Reiner, 2000)6.

Interferon-gamma(IFN-γ)

Interferon-gamma, mainly produced by activated CD4 and CD8 T-cells, and NK cells, is a homodimeric glycoprotein having two subunits each of 21 to 24KDa. IFN-γ is a potent activator of mononuclear phagocytes inducing the respiratory burst mechanism. IFN-γ also up regulates the

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expression of MHC class II molecules, leading to amplification the process of CD4 T-cell activation and promotes their differentiation to Th1phenotype, and inhibits the proliferation of the Th2 type cells and thus tends to suppress humoral immunity. It not only decreases the production of IL-4 by Th2 cells but also potently inhibits the effects of IL-4 on B cells, particularly inhibiting IgE production. GKO mice, in which IFN-γ or IFN-γ receptor genes have been disrupted, show several immunological defects including impaired macrophage activation, reduced NO production, reduced MHC class II molecules expression, reduced IgG2a and IgG3 serum levels and defective NK-cells function (Benjamiini, *et al.*,2000)5. IFN-gamma is the primary lymphokine responsible for stimulating macrophage oxidative burst and activating anti infectious mononucleosis activity in vitro (Murray and Delph-Etienne,2000)7. IFN-gamma also induces the secretion of TNF-alpha upon activation of macrophages for intracellular killing of the infection by enhancing NO production (Bogdan *et al.*, 1998)8. The efficacy of IFN-gamma was tested by gene transfer experiments in which injection of a mammalian expression plasmid bearing IFN-gamma gene in normal and in IFN-gamma gene disrupted infected mice led to controlled visceral leishmaniasis infection and reduced parasite burden (Taylor and Murray, 1998)9.

Materials and Methods

Subjects

Patient's study group Patient's groups included in this study could be devided as follows.

A total of 100 patients were subjected to this study. These patients with presumably clinical picture (fever, lymphadenopathy, pharyngitis and atypical lymphocytosis) of infectious mononucleosis. They were referred to central puplic health laboratory (CPHL). Sixty five of them(The range of age 4-40years) were send to CPHL from different Hospitals.

Blood samples from patients with suspected of infectious mononucleosis –like illness were chosen for sampling and were investigated there in Hematology, Virology, Serology and immunology departments. In addition to the groups, 30 control blood samples (the range of age 4-39years) were collected from normal persons attending the CPHL for the purpose of obtaining Health Certification.

Kit used in this study Interferon-gamma enzyme immunoassay by Immunotech A Beckman Coulter company , France.

Method

Enzyme immunoassay for Quantitative Determination of human Interferon-gamma in serum.

A-Principle

The immunoenzymatic assay of interferon-gamma (IFN-γ) is sandwich type assay with two immunological steps. The first step leads to capture IFN-γ present in the sample by monoclonal anti IFN-γ, antibody bound to the wells of microtiter plate. In the second step, a second monoclonal anti-IFN-γ antibody, which is biotinylated is added together with streptavidin-peroxidase conjugate. The biotinylated antibody is bound to the solid phase antibody-antigen complex and in turn, binds the conjugate. After incubation period, the wells are washed and the binding of the streptavidin-peroxidase via biotin is followed by the addition of chromogenic substrate of the peroxidase.

Procedure, according to the information supplied by Immunotech A Beckman Coulter company, as mentioned below:

B-Immunoassay procedure

1.One well in the microtiter plate was left empty for substrate blank.

2.To antibody coated wells, 50µl of standard (1000, 500, 250 ,62.5, 3.9 and 0) pg/ml or sample was added and the plate was incubated for 2 hours at(18-25C°) with shaking at 350rpm.

3.The plate was washed3 times using microiter plate washer.

4.Fifty µl of biotinylated antibody and 100 µl of streptavidin HRP solution were added, and the plate was incubated for 30 minutes at (18-25C°) with shaking at 350rpm.

5. The plate was washed 3 times using microiter plate washer.

6.Hundred µl of the substrate was added and the plate was incubated for 15minutes at(18-25C°) with shaking at 350rpm in the dark.

7.Fifty µl of stopping solution was added into all wells and the absorbance was read at 450nm.

C- Calculation of the results.

The standard curve was drawn by plotting on the horizontal axis the IFN-γ concentrations of the standards and on the vertical axis the corresponding average absorbance. To locate the concentration of IFN-γ in the samples, the average absorbance for each sample on the vertical axis was located and the corresponding IFN-γ concentration was located on the horizontal axis.

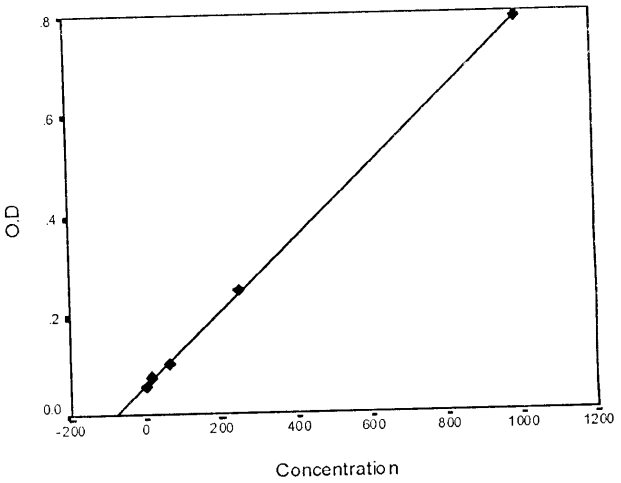
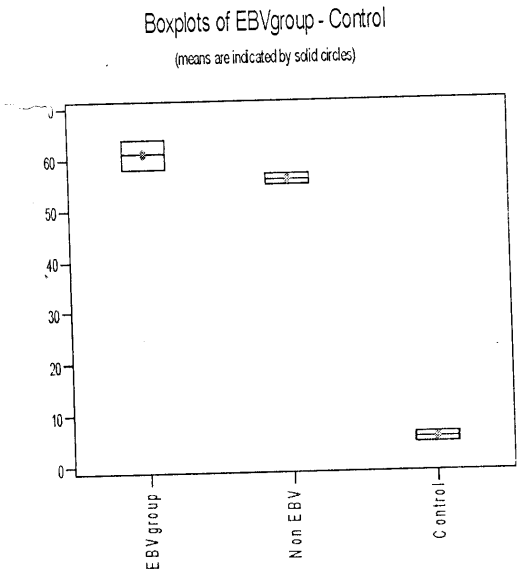


Figure (1):Standard curve of IFN-gamma

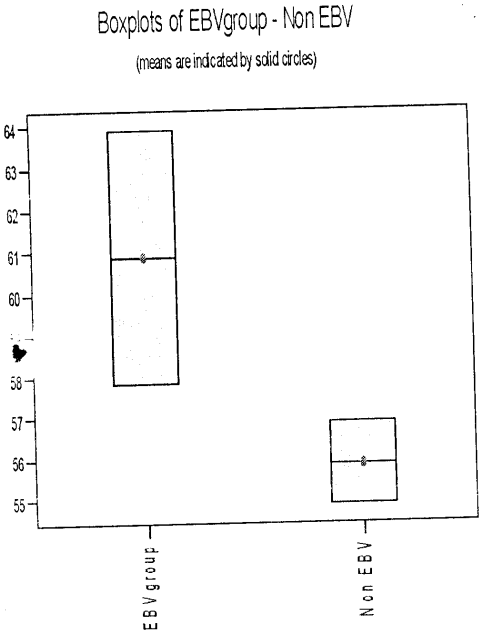
Results

Serum IFN-gamma level

Serum level of IFN-gamma in the control were(5.12±0.8) pg/ml significantly lower than that in patients with EBV 60.87±2.498pg/ml. In the Non EBV infection the levels were (55.850±0.825) pg/ml which were significantly lower than patients with EBV groups (<0.001).There was high significant difference between EBV, Non EBV and Control groups figures(2,3 and4)



Figure(2) serum levels of IFN-gamma measured by ELISA in EBV, Non EBV and control groups



Figure(3) :Serum levels of IFN-gamma, measured by ELISA in patients of EBV and Non EBV groups

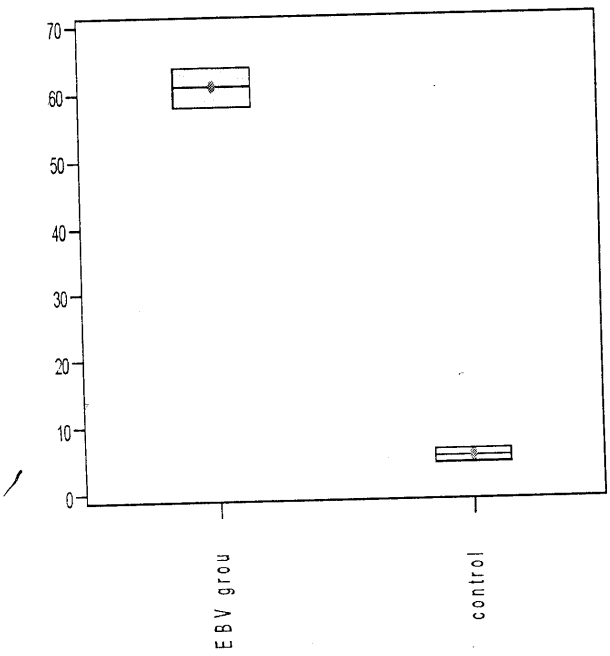


Figure (4):Serum levels of IFN-gamma, measured by ELISA in patients of EBV and Healthy control groups

Discussion

High levels of IFN-gamma have been detected in serum of patients with infectious mononucleosis due to infection with EBV in comparison with patients of IM due to non EBV infection and Healthy control groups. High levels of IFN-gamma are necessary in the maintenance balance between Th1 and Th2 responses.

These results matched the results of previous researchers (Gomes and Dos1998)9 who was found a mixed Th1/Th2 response of parasite-specific T-cells from both acute and chronic murine visceral leishmaniasis (Kemp and Theander,1999)10 stated that marked elevation of both IL-10 and IFN-gamma mRNA found in patients with IM. Our study agrees with other studies (Ohga,*et al.*;2004)11 who suggested that EBV T-cells are activated to transcribe IFN-gamma,IL-10, these two genes are expressed preferentially in the imply a viral evasion mechanism in the disease .

A different observation showed that in American infectious mononucleosis , the IL-2 and IFN-gamma production were increased upon stimulation with IM. Also Bogden *et al.*(1991)12 noted that IL-10 blocks Th1 activation and consequently a cytotoxic response by down-regulating IL-12 and IFN-production, IL-10 also inhibits macrophage activation and decreases the ability of these cells to kill IMS

Conclusion.

High levels of IFN-gamma may be referred to a mixed TH1/TH2 response in infectious mononucleosis –like syndrome.

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