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Evaluation the concentration of interferon- gamma and, GM-CSF in patients with tonsillitis

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Summary

This study was conducted on 90 subjects whom have visited the AL-Sadder Medical City and Al-Hakeem hospital in AL- Najaf governorate between the period extended from February to August, 2013. Subjects of this study were seventy chronic or recurrent tonsillitis patients, their age ranged from: 4-33 years and comprised of 29 males and 41 females.. In addition, 20 (age-and sex-matched) healthy adults without any evidence of chronic inflammatory disease depended as the control group.

Five milliliters of blood sample collected from each patient , for the purpose of obtaining a serum sample and measured the concentration of cytokines using ELISA technique . Results showed a significant increase to the high concentration of cytokines : interferon - gamma , colony stimulating factor granule cells and mononuclear , in all the groups of patients compared with those of healthy control group

1. Introduction

Tonsillitis is the inflammation of the tonsils as a result of infection mostly caused by a virus especially the Epstein-Barr virus Adenoviruses, influenza virus, para influenza virus and Enteroviruses and is often preceded by a cold (a runny nose, cough and sore throat). Fewer cases (about one in seven) are caused by bacteria [1].

The tonsils are an important part of the immune system. They are two lymphatic glands located on the back part of the throat. The tonsils are also called the palatine tonsils and they can clearly be seen when a person opens up their mouth wide enough for them to be revealed [2].

Cytokines are vital in host defence against viral infection, with complex networks and pathways [3].

Gamma interferon (IFN- γ) was a highly pleiotropic protein secreted mainly by activated T-lymphocytes and natural killer cells. It is involved in a wide range of physiological processes, including antiviral, immunoregulatory and anti-tumour properties, cell proliferation and apoptosis, as well as the stimulation and repression of a variety of genes [4].

Granulicyte Monocyte – Colony stimulation factors (GM-CSF) is a small glycoprotein growth factor, a pleiotropic cytokine that can stimulate the proliferation, maturation and function of hematopoietic cells. It is produced by a variety of cell types including T cells, B cells, macrophages, mast cells, endothelial cells and fibroblasts in response to cytokine or immune and inflammatory stimuli.

The current study sets an objective to determin the serum level of cytokines: IFNγ, and GM-CSF in patients with tonsillitis to role out any immune deficiency.

2-Materials

2.1 Subjects

Sadder medical city and Al-Hakeem hospital in AL-Najaf city between Februarys to August 2013, they were (29 males and 41 females) and their ages ranged between (4-33 years) age. physical examination showed exudative tonsillitis accompanied by exudative and/or erosin of the oropharyngeal mucosa and cervical lymphadenopathy, were investigated from.

Control group is comprised of (20) healthy subjects (9 males and 11 females) without any history of hematologic, recurrent upper respiratory tract infection or inflammatory diseases and hypertrophy of tonsils.

Blood sample were taken 24 hr. prior to tonsillectomy (pre-operative) a volume of 5ml of venous blood was withdrawn from each patient under study, were left to coagulated and centrifuged at 300 r.p.m and the serum were aspirated to another glass test tube .

Cytokine detection

The cytokines (Interferon-γ, GM-CSF, were measured using Enzyme-Linked Immunossorbant Assay at the Virology department in Al-Sadder medical city in AL- Najaf governorate as follow.

Interferon gamma (IFN-γ):

The AssayMax Human IFN-γ ELISA kit was performed according to the manufacturing company (Assaypro, U.S.A) as follows:

1. The components of the kits were equilibrated at room temperature before use.
2. The components of the kits were prepared as follows:

Reagent	Preparation
Human IFN-γ Microplate	96 wells ready to use
Human IFN-γ - Standard (2 ng/ml)	Adding 2ml of EIA Diluent to generate a 1ng/ml of solution
Biotinylated IFN-γ – Antibody	Dilute 0.08ml in 8ml MIX Diluent
EIA Diluent Concentrate	Dilute 30ml in 300ml of D.W.
Wash Buffer Concentrate	Dilute 30ml in 600ml of D.W.
Streptavidin-Peroxidase Conjugate	Dilute 0.09ml in 9 ml MIX Diluent
Chromogen Substrate	8ml ready to use
Stop Solution (0.5 N HCL)	12ml ready to use

3. The standard solution and the appropriate diluents were prepared as follows:

Standard Point	Dilution	[IFN-γ] C0NCENTRATION (ng/ml)
P1	Standard (1 ng/ml)	1.000
P2	1 part P1 + 1 part EIA Diluent	0.500
P3	1 part P2 + 1 part EIA Diluent	0.250
P4	1 part P3 + 1 part EIA Diluent	0.125
P5	1 part P4 + 1 part EIA Diluent	0.063
P6	1 part P5 + 1 part EIA Diluent	0.031
P7	1 part P6 + 1 part EIA Diluent	0.016
P8	EIA Diluent	0.000

Procedure:

- Fifty µl of Standard (8), controls (20) and samples (70) were added per well. Wells were covered with a sealing tape and incubated for two hours. The timer was started after the last sample addition.
- The microplate was washed six times with 300 µl /wells of Wash Buffer using bioeliser washer ELx 50 (biokit, U.S.A).
- Fifty µl of Biotinylated anti IFN-γ antibody were added to each well and incubated at room temperatures for two hours.
- The microplate was washed as described above.
- Fifty µl of Streptavidin-Peroxidase Conjugate were added per well and incubated at room temperatures for 30 minutes. The bioelisa reader ELx 800 (biokit, U.S.A) was turned on and set up the program in advance.
- The microplate was washed as described above.
- Were µl of Chromogen Substrate were added per well and incubated at room temperatures for about 15 minutes or until the optimal blue color density develops.
- Fifty µl of Stop Solution were added to each well. The color will change from blue to yellow. The absorbance on bioelisa reader ELx 800 was read at a wavelength of 450 nm immediately. Results were provided within 1 minute on the LCD display.

Calculation of results:

Results were calculated using standard concentration curve. The standard curve measures the relationship between optical density (O.D) and standard (ng/ml) and the results were interpolated using Graphpad prism. The correlation coefficient (r) was 0.8998.

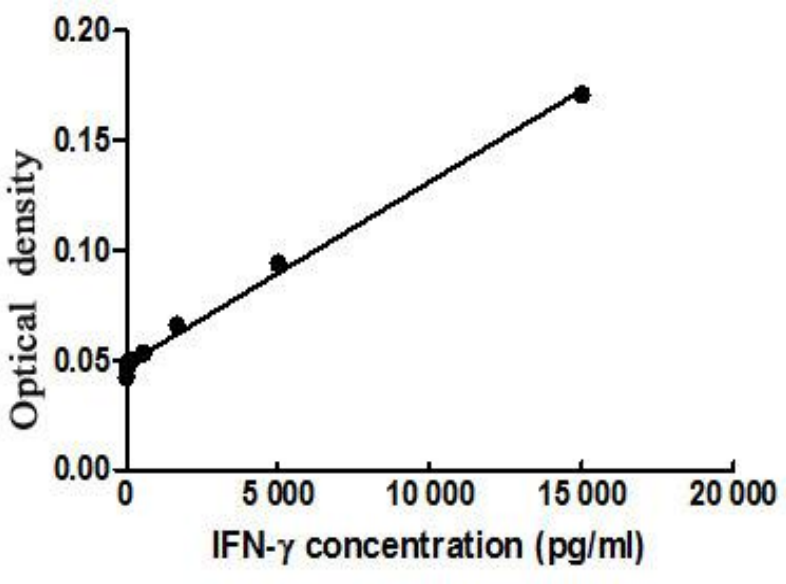

Fig (1-1): IFN-γ standard curve.

Granulocyte, monocyte- colony stimulation factors (GM-CSF):

The RayBio Human GM-CSF ELISA kit was performed according to the manufacturing company (RayBiotech, Inc. U.S.A) as follows:

1. The components of the kits were equilibrated at room temperature before use.
2. The components of the kits were prepared as follows:

Reagents	Preparation
GM-CSF Microplate	96 wells ready to use
Wash Buffer concentration	Dilute 20ml in 400ml of D.W.
Assay Diluent Antibody GM-CSF	Dilute 6 ml in 24 ml D.W.
Human GM-CSF standards(100ng/ml)	Adding 5µl of GM-CSF standard to tube with 995 µl of Assay Diluent to generate a 500pg/ml of solution
Biotinylated Antihuman GM-CSF	Adding 100 µl of Assay Diluent into the vial then mix solution with 12 ml Assay Diluent
HRP-Streptavidin concentrate	Adding 2 µl of HRP-Streptavidin concentrate in to tube with 198 µl Assay Diluent and then pipette 60 µl in to tube with 6 ml Assay Diluent.
TMB One-Step Substrate Reagent	12ml ready to use
Stop Solution(0.5 N HCL)	8 ml ready to use

3. The standard solution and the appropriate diluents were prepared as follows:

Standard point	Dilution	GM-CSF concentration (Pg/ml)
P1	Standard (500 pg/ml)	500.0
P2	1 part P1 + 1.5 part Assay Diluent	200.0
P3	1 part P2 + 1.5 part Assay Diluent	80.0
P4	1 part P3 + 1.5 part Assay Diluent	32.0
P5	1 part P4 + 1.5 part Assay Diluent	12.80
P6	1 part P5 + 1.5 part Assay Diluent	5.12
P7	1 part P6 + 1.5 part Assay Diluent	2.05
P8	Assay Diluent	0.00

Procedure:

- One hundred µl of Standard (8), controls (20) and samples (70) was added per assigned well. Wells were covered with a sealing tape and incubated for 2.5 hours at room temperature with gentle shaking. The timer was started after the last sample addition.
- The microplate was washed four times with 300 µl of Wash Buffer/well using bioeliser washer ELx 50 (biokit, U.S.A)
- One hundred µl of Biotinylated anti GM-CSF antibody were added to each well and incubated for one hour at room temperature with gentle shaking.
- The microplate was washed as described above.
- Streptavidin solution (One hundred µl) / per well were added and incubated for 45 minutes at room temperature with gentile shaking. The microplate was washed as described above.
- TMB One-Step Substrate Reagent (One hundred µl) were added to each well and incubate for 30 minutes at room temperature in the dark with shaking. The bioelisa reader ELx 800 (biokit, U.S.A) was turned on and set up the program in advance.
- Stop Solution (fifty µl) were added to each well. The color will change from blue to yellow. The absorbance on bioelisa reader ELx 800 was read at a wavelength of 450 nms immediately. Results were provided within 1 minute on the LCD display.

Calculation of results:

Results were calculated using standard concentration curve. The standard curve measures the relationship between optical density (O.D) and standard (pg/ml) and the results were interpolated using Graphpad prism. The correlation coefficient (r) was 0.9558.

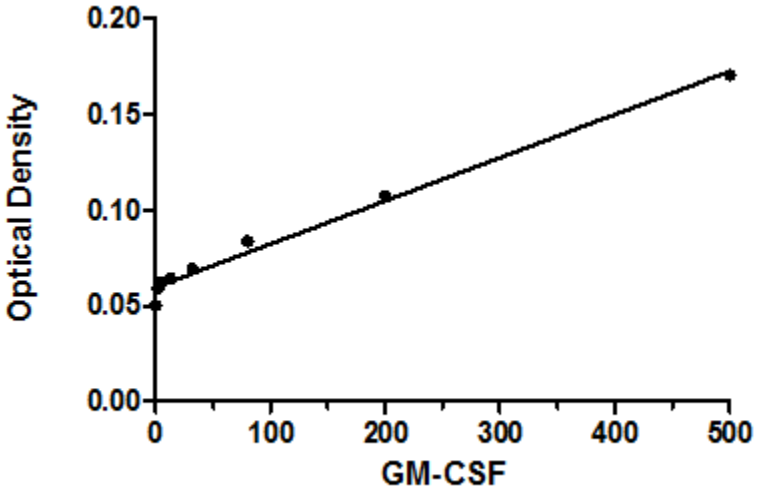

Fig 1-2: GM-CSF standard curve.

RESULT

INF-γ

The study showed a higher significant increase ($p < 0.0001$) in the level of INF-γ in all groups of patients suffering from chronic or recurrent tonsillitis than the healthy control group. However it's less significant in D-1 and D-4 (Figure 1-3)

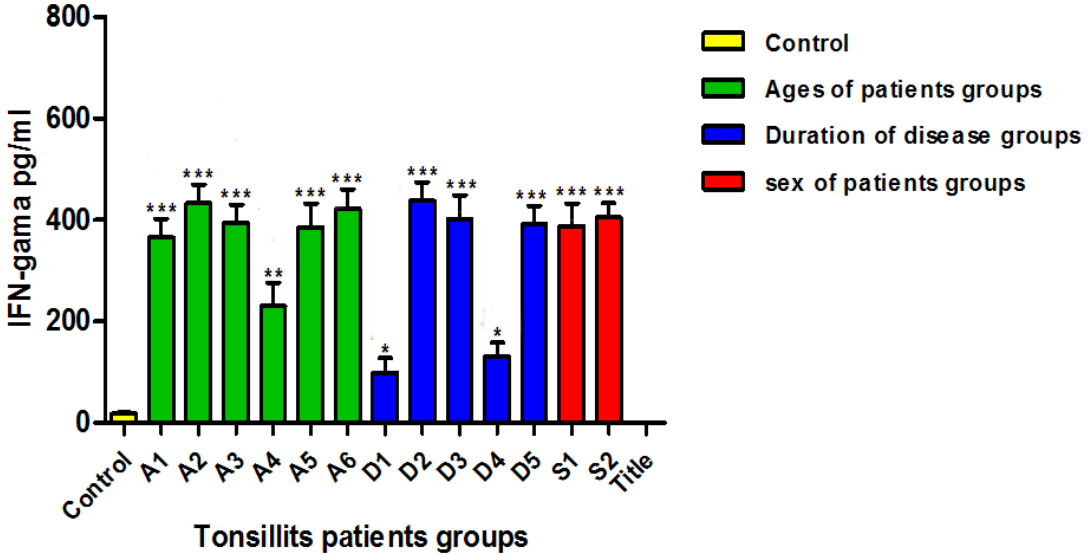

Figure (1-3): Level of INF-γ in all studied groups of tonsillitis patients compared with healthy control group.

- Data are expressed as mean ± standard error (SE)
- The asterisks indicate significant difference compared to control based on Tukey's multiple comparison test.
- Ns: not significant.

- A1: the group of patients with the age (<5) years.
- A2: the group of patients with the age (6-10) years.
- A3: the group of patients with the age (11-15) years.
- A4: the group of patients with the age (16-20) years.
- A5: the group of patients with the age (21-25) years.
- A6: the group of patients with the age (> 26) years.
- D1: the group of patients with disease duration of (<1) years.
- D2: the group of patients with disease duration of (2-3) years.
- D3: the group of patients with disease duration of (4-5) years.
- D4: the group of patients with disease duration of (6-7) years.
- D5: the group of patients with disease duration of ((> 8) years.
- S1: female group.
- S2: male group.

GM-CSF

According to the result of our study there was increase in level of GM-CSF in all groups of patients suffering from tonsillitis in comparison to the healthy control group and this increase was statistically significant in all groups (Figure 4-8).

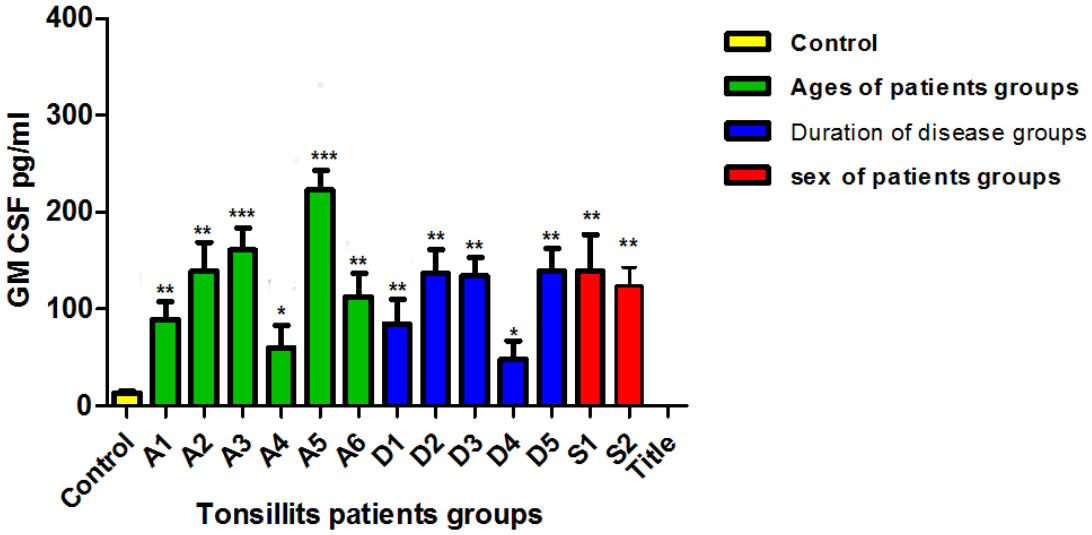

Figure (1-4): Level of GM-CSF in the studied groups compared to healthy control group.

- Data are expressed as mean ± standard error (SE)
- The asterisks indicate significant difference compared to control based on Tukey's multiple comparison test.

4- DISSCUSION

. Serum Levels of IFN-γ

The current study revealed that the serum concentrations of IFN-γ were significantly elevated in tonsillitis patients compared to those in healthy control group as seen in previous reports.

IFN-γ was originally discovered because of its ability to induce cell to block or inhibit the replication of a wide variety of viruses and play a central role in many immunoregulatory processes including mononuclear phagocyte, B cell switching to certain IgG and inhibition of Th-cell subset.

Emphasizing the role of IFN-γ as a major cytokine in infection mononucleosis. High levels of IFN- γ are expressed by TH1cells, activating macrophages to kill microbes, promoting cytotoxic activities of other cells, and inducing apoptosis of epithelial cells in the skin and mucosa [5].

The current study exhibited that INF-γ was commonly detectable in all cases of tonsillitis. Together with detectable cytokines, namely, IL-4, IL-1α, IL-10, these cytokines correlate with EBV infection.

Serum Levels of GM-CSF

Serum levels of GM-CSF were significantly elevated in all groups of patient with tonsillitis compared with healthy control group.

GM-CSF may stimulate the hematopoietic regeneration and the functional activity of granulocyte and monocyte, GM-CSF substantially enhanced the antiviral activity thus improving the immune status of patients which is implicated in the pathogenesis of the disease.

[6]. reported that the elevation of GM-CSF IFN-γ, IL-4, and IL-10 may also be responsible for the clinical symptoms in chronic IM caused by EBV.

However, [7]. reported that increase secretion of GM-CSF, ILI-α, IL-4, INF-γ, and IL-10 with EBV infection of tonsils, during the initial response to EBV infection, the innate immune response is capable of suppressing viral replication more efficiently than is the adaptive immune response.

5- Conclusion:

From this study we can conclude the following;

1. The serum levels of proinflammatory cytokines IFNγ, GM-CSF, play a major role in the pathogenesis of tonsillitis, as part of the immune defense.

2. The association of IFNγ, and GM-CSF its effect on different component of cell mediated immunity by increasing or decreasing according to site of activity and other environmental factors.

6- Reference:

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