Evaluation of cellular immune response in patients with cytomegalovirus by monitoring the serum levels of Granzymes A ,B and GM-CSF

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Abstract:

Human Cytomegalovirus (CMV) is a ubiquitous opportunistic pathogen in immunosuppressed individuals. Granzymes are a unique serine proteases which plays a crucial role in target cell death, this substances produced by NK cells and CTL in part of the cellular immunity. This work was curried in the period between March and August 2007, the aim of this work was evaluation the cellular immunity against CMV chronic infection by determination the levels of serum granzymes and GM-CSF in seropositve individuals. Obtained results declared a significant increment in the levels of granzyme A and B. Levels of granzyme A documented a clear increase 112.33 ± 22 pg/ml in comparison to control group which was 21.11± 12 pg/ml. Also Levels of granzyme B recorded a significant increase 77.11 ±17 pg/ml in comparison to control group which was 18.11 ± 9.1 pg/ml. GM-CSF concentrations showed a remarkable increment in the serum of the patients about 132.66 ± 33 pg/ml in comparison to control group which record 42.59 ±27 pg/ml. Phagocytosis activity recorded no or very slight, non significant changes in patients group in comparison to control group.

These results may suggest that, the cell mediated innate and specific immunity is crucial for controlling the CMV infection and GM-CSF have a protecting role by enhancing the proliferation of leukocytes, which is important in controlling the infection and make it asymptomatic.

Introduction :

Cytomegalovirus (CMV) is a member of herpes virus family, this virus associated with persistent , latent and recurrent infections (1) . The infection with this virus is relatively common 60-90% of community and usually asymptomatic in healthy immunocompetent individuals the virus persist in a latent state throughout the life of the host, under the control of cell-mediated immune surveillance (2,3). CMV remains latent in monocytes , granulocyte-monocyte progenerator cells and perhaps in other cell types especially immunosuppressed individuals(3). Infection with this virus can result in a variety of clinical syndromes, depending partially on the immune status of the infected individual (4). Patients with ineffective cellular immunity are at high risk to CMV infection (5). Cells such as natural killers (NK) and cytotoxic T lymphocytes (CTL) use cytoplasmic granules containing perforin and granzymes to kill the target cells by their ability to lyses certain tumor and virally infected cells (2,6,7) .

Recent study suggest that CMV initiate innate immune response through the activation of Toll-Like receptors (TLR), this receptors make NK able to sense viral pathogens directly, and monitoring the level of class I major histocompatibility complex (MHC) molecules expressed on the cells (7).

CTL lysis of CMV infected cells occur primarily through granule exocytosis, which require perforin to facilitate the entry of apoptosis-inducing serine protease granzymes into the cytosol of the infected cell (8,9).

Granzymes, a family of related serine proteases, during the target cell recognition, the continent of these granules are directionally released toward the target cell, then perforin molecules form 10-20 nm pores in the cytoplasm membrane of these cells, perforin are not sufficient to kill nucleated cells and these pore appeared to function as means of delivering granzymes into target cell, granzymes triggers apoptosis in this cells (6,7,9). Recent studies suggested that granzymes expression can be used as a marker for CTL capable of cytotoxicity in cases of transplant rejection and viral infections. Serum levels of granzymes were known to be increased in patients with primary Cytomegalovirus which can induce expression of several transcription factors responsible of production various types of cytokines (10,11,12). One of the important cytokines is the Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), one of colony stimulating factors, and is a hemoatopietic growth factor and is a member of hematopietin family (13). GM-CSF principle sources is the stromal cells and activated

macrophages, it is a pleiotropic cytokine that exhibit effects on most cell types by promoting and supporting the proliferation of both multipotential and precursors committed to monocyte and neutrophils formation. This cytokine was used in clinical trials in hemoatopietic recovery of certain hematological diseases and after some chemotherapy courses which may impair the immunohematological competent cells (14,15) .

Materials and methods :

Detection of viral infected patients :

This search was curried in the period between June and August 2007**,** the patients group included 37 chronic asymptomatic seropositve Iraqi patients with cytomegalovirus, and 23 healthy seronegative individuals were randomly selected as control group .

Detection of Viral Marker :

ELISA kit was used for Detection of biotnylated anti-IgG monoclonal antibodies to Cytomegalovirus, as recommended by manufacturing (MabTech. Austuria) .

Determination the concentrations of Granzymes A and B :

Serum concentrations of granzymes A and B ,were measured by means of enzyme linked immunosorbent assay using specific ELISA kits, Measurement done as recommended by the manufacture (Cell Sciences Inc. USA).

Determination of phagocytosis:

Phagocytosis activity were tested in both patients and healthy control group (15) .

Determination the concentrations of GM-CSF:

ELISA kit was used for Detection of biotnylated monoclonal antibodies, as recommended by manufacturing (MabTech. Austuria) .

Results :

Obtained results showed a remarkable increment in the serum concentrations of granzymes A and B . Granzyme A levels (mean ± SD) 112.33 ± 22 pg/ml while in control group 21.11± 12 pg/ml (figure 1).

Granzyme B concentrations (mean ± SD) also recorded a clear increase 77.11 ±17 pg/ml in comparison to control group 18.11 ± 9.1 pg/ml (figure 2).

GM-CSF concentrations showed a remarkable increment in the serum of the patients about 132.66 ± 33 pg/ml in comparison to control group which record 42.59 ±27 pg/ml.

Phagocytosis activity recorded no or slight non significant changes in patients group in comparison to control group.

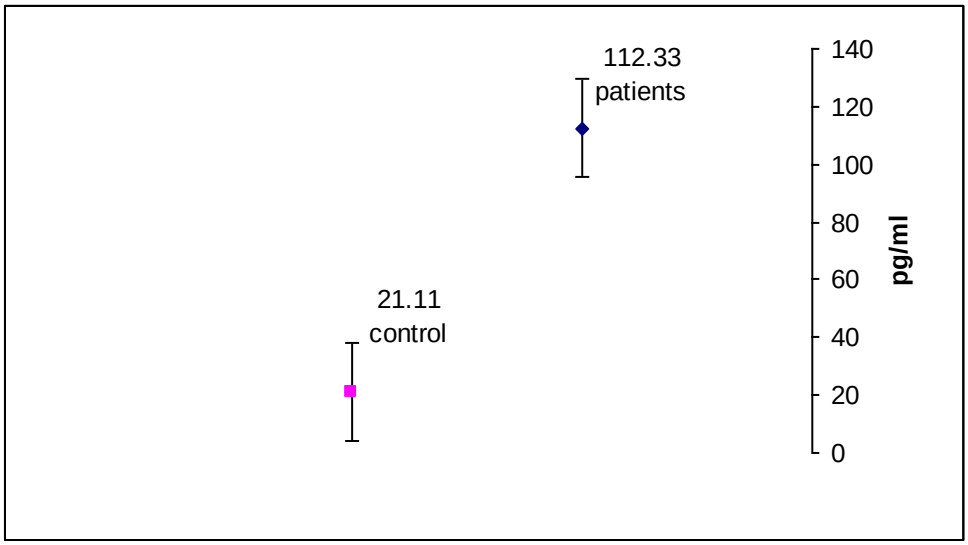

Figure-1: Serum levels of Granzyme A in patients with CMV.

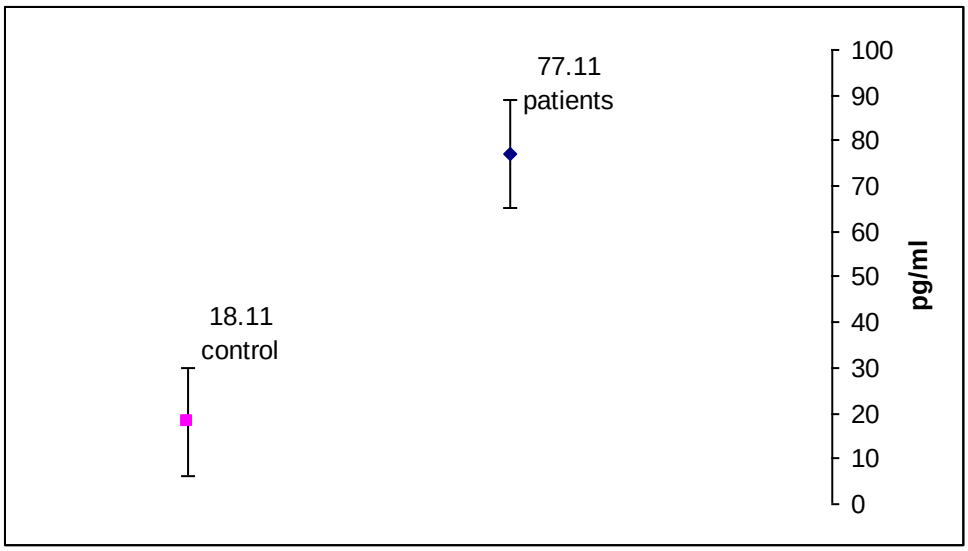

Figure-2: Serum levels of Granzyme B in patients with CMV.

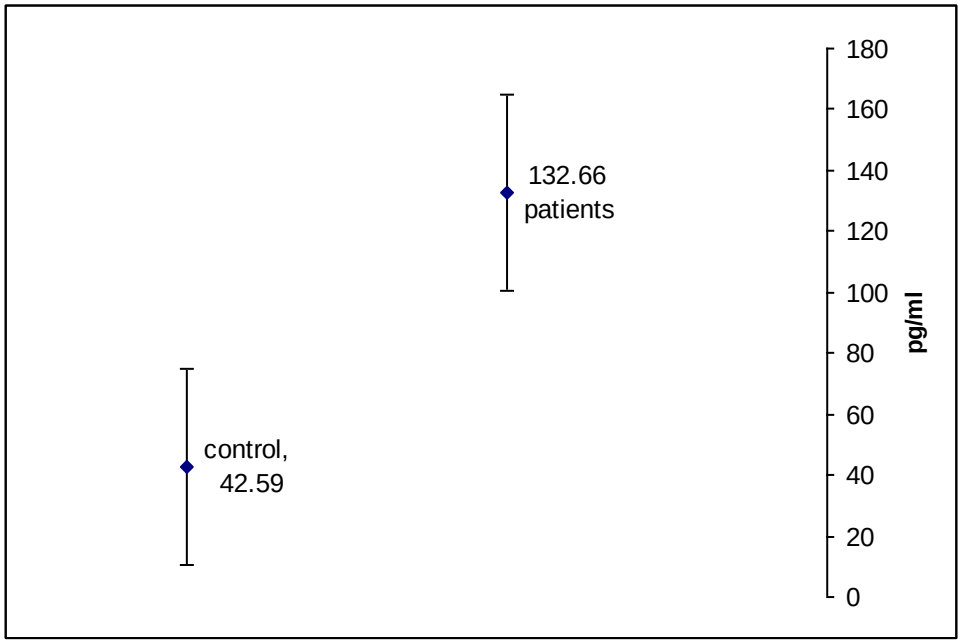

Figure-2: Serum levels of GM-CSF in patients with CMV.

Discussion :

Human Cytomegalovirus (CMV) is a ubiquitous opportunistic pathogen. Clinically ,CMV disease correlates with immune suppression. Histological analysis of autopsy tissues obtained from patients with CMV has demonstrated infected cells in virtually all organs. At the cellular level CMV can infect monocytes /macrophages, endothelial cells, smooth muscle cells, fibroblasts, stromal cells, epithelial cells, neutrophils, and hepatocytes(12,16).

Structural components of CMV causes a cereus alterations in cellular transcription factors, and as a result, production of several types of immune mediators and cytokines such as IL-7, IL-8, IL-11, IL-6, IL-2, TNF-α and IFN-γ. Also CMV infection influence the expression of several chemokines (13,17).

Cytotoxicity of target cell is a promising and an effective mean for the elimination of tumor and viral infected cells. Cytotoxicity activity usually determined by a complex interfered signals of the immune system components (18).

Most antiviral CD8 T cells during chronic viral infections do not express high levels of perforin and are not directly cytotoxic (19).

Recent studies documented a remarkable increase of soluble granzyme B due to emergence of $CD4^+$ CD^- GrB^+ cytomegalovirus specific subset T cells after recovery of primary cytomegalovirus infection (10). Another studies suggested that granzyme B could be used as a marker of primary cytomegalovirus infections in renal transplantation (20).

Cytotoxic $CD4^+$ $CD8^-$ T cells appeared in circulation only after cessation of viral replication, and are detectable in much higher frequencies in CMV-seropositve individuals during latency of the virus, also the increase of cytotoxic T cells in peripheral blood compartment is only after the viral load become undetectable , that's explain the entry of the CTL into the circulation from infected tissues only once the acute infection is over (21,22).

Results of this work showed a remarkable significant increment in levels of two types of granzymes A and B and come in conformity with the previous studies. This increase my be explained by the emergence of cytotoxic T cells and the increase of the activity of natural killers in peripheral blood, in part of innate and specific immunity components.

Previous recent studies indicated the involvement of NK cells as innate immunity resistance during infection with malaria through increment of soluble granzymes A and B in serum in the patients (23).

In this work GM-CSF levels record a significant increment, indicating that the viral infection caused a recordable changes of this cytokine in the patients sera, this suggestion come in conformity with the previous studies on other types of cytokines.

Recent study used GM-CSF as recombinant drug which approved for hematological indications in human, and enhance the synthesis and release of many proinflammatory cytokines such as TNF, which is crucial for host defense against infectious agents(13, 24,25). In recent study in Iraq, GM-CSF was imbalanced in some viral infections such as viral hepatitis C (26).

In this work, the phagocytosis appeared as normal, and it was statistically not significant, the increase of GM-CSF levels may be the real protecting cause of the normal blood phagocytosis activity in the patients group, which appeared non affected in comparison to control healthy group.

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تقييم الاستجابة المناعية الخلوية في المرضى المصابين بفيروس Cytomegalovirus من خلال متابعة التركيز المصلية لكل من A Granzymes وB وGM-CSF

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الخلاصة:

إن فيروس CMV هو فيروس ممرض انتهازي عالمي الانتشار في الأشخاص المثبطين مناعياً. كذلك فان Granzymes هي مجموعة مميزة من إنزيمات serine proteases التي تلعب دوراً مهما في عملية قتل الخلية الهدف، وتفرز هذه المواد من قبل كل من خلايا القاتلة الطبيعية NK cells والخلايا اللمفاوية السمية CTL cells أثناء عملية الاستجابة المناعية الخلوية. اجري هذا البحث في الفترة بين شهري آذار وشهر آب من عام 2007، بهدف تحديد الاستجابة المناعية الخلوية عند المرضى بفيروس CMV بشكل مزمن، وذلك من خلال دراسة تركيز Granzymes و GM-CSF في أمصال الأشخاص الذين أعطوا فحصاً موجباً لوجود أضداد الفيروس. أوضحت النتائج التي تم الحصول عليها وجود ارتفاعاً معنويا في قيم تركيز Granzymes في المرضى عما هو عليه بمجموعة السيطرة الأصحاء، حيث بلغ متوسط تركيز النوع A 112.33 ± 22 pg/ml عما هو في مجموعة السيطرة 21.11± 12 pg/ml. وكذلك للنوع B الذي سجل هو الآخر زيادة معنوية بلغت 77.11 ±17 pg/ml مقارنة بمجموعة السيطرة التي سجلت18.11 ± 9.1 pg/ml. كذلك بينت الدراسة وجود ارتفاع معنوي واضح بتراكيز GM-CSF في مجموعة المرضى حيث بلغت 132.66 ± 33 pg/ml مقارنة بمجموعة السيطرة التي كانت 42.59 ±27 pg/ml. كما وشملت الدراسة فحص قابلية الخلايا الدموية البيضاء على البلعمة، حيث سجلت النتاج عدم وجود اختلافات معنوية واضحة وان وجدت لم تكن معنوية إحصائيا في مجموعة المرضى مقارنة بمجموعة السيطرة الأصحاء.

إن هذه النتائج تؤكد على أهمية آليات الاستجابة المناعية الخلوية في السيطرة على الإصابة بفيروس CMV ، كما وتشير إلى الدور الذي يلعبه GM-CSF في تحفيز تضاعف الخلايا الدموية البيضاء، التي بفضل دورها تتم السيطرة على المرض الفيروسي وبقائه بصورة دون السريرية.