British Journal of Medicine & Medical Research
4(3): 883-888, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Effect of Cytomegalovirus and Diabetes Mellitus on the Cytokine Profile of Cellular Immunity in Tuberculosis Patients

Mohammed A. K. AL-Saadi[1*], Jawad K. AL-Khafaji[1] and Nazar A. Sheriff[1]

[1]Department of Medical Microbiology, College of Medicine, Babylon University, Hilla, Iraq.

Authors' contributions

This work was carried out in collaboration between all authors. Author MAKA participated in study design and wrote the draft of manuscript, Author JKA participated in study design and carried out the statistical analysis. Author NAS collected and processed the samples. All authors read and approved the final manuscript.

Original Research Article

Received 30th August 2013
Accepted 10th October 2013
Published 23rd October 2013

ABSTRACT

Aims: This study aims to investigate the effect of cytomegalovirus (CMV) and diabetes mellitus (DM) on the cell-mediated immunity against TB represented by cytokine profile
Study Design: Case control study.
Place and Duration of Study: This study was carried out in Specialized Chest and Respiratory Center in Baghdad, Iraq and Department of Medical Microbiology-College of Medicine -Babylon university Hilla-Iraq, the period of study was October 2012 to January 2013.
Methodology: This study was applied on 70 TB patients .It involved also 30 apparently healthy control. The patients consists of 43 males and 27 females with age range 8-76 years old, 29 of them were diabetic .Blood samples were collected from patients and controls to estimate the immune parameters interferon-gamma (IFN-γ) and interleukin -2(IL-2)as , and anti –CMV IgG antibodies by enzyme linked immunosorbent assay (ELISA).
Results: The immune parameters showed that there is no significant difference in the mean serum concentration between the patients and control groups for IFN-γ and IL-2 (P=0.05), while there was a significant increase in the mean serum concentration of anti-CMV IgG between study groups (P≤0.001).

**Corresponding author: Email: mbmc.kadhum70@gmail.com;*

The study also showed that there is a significant decrease in the mean serum concentration of IL-2 and IFN-γ between diabetic TB patients comparing with those non-diabetic TB patients where p values were 0.008 and 0.024 respectively.
Conclusion: Both CMV and diabetes mellitus have a role in the suppression of cellular immunity in TB patients.

Keywords: Tuberculosis; cytomegalovirus; anergy; diabetes mellitus.

1. INTRODUCTION

Tuberculosis (TB) remains a major global health problem. Ten million new TB cases and 2 million deaths are estimated to occur each year, more than any time in history [1]. Furthermore, an estimated 2 billion people are thought to be latently infected, providing a large reservoir for active TB that will last for decades [2]. Tuberculosis is considered an immunological disease and the pathology of the disease is mediated by the host immune response [3].Cell mediated immunity is the effective mechanism against tubercle bacilli and the T_{H1} cytokines mainly interferon gamma (IFN-ɣ) and interleukin-2(IL-2) play a central role in the activation of mycobactericidal activity of alveolar macrophages [2,3].

Immune anergy as a mechanism of peripheral tolerance may increase the severity of TB [4]. Many predisposing factors increase the frequency of immune anergy including viral infections and metabolic disorders especially renal failure and diabetes mellitus [5]. Cytomegalovirus (CMV) is a ubiquitous beta-herpes virus with a worldwide prevalence of 60-100% in the adult population [6]. Infection occurs early and leads to life-long persistence in the host. CMV is one of the most immunodominant antigens and stimulates immune responses of unprecedented magnitude [7]. Several studies have shown that latent infection with cytomegalovirus contributes to age-related alterations of the immune system and immune anergy, particularly of the T cell compartment as it drives the differentiation of T cells and accelerates immunosenescence [6].

Diabetes Mellitus (DM) is an epidemic worldwide and it is estimated to affect 366 million by 2030 when majority of those affected will be living in low and middle income countries where Mycobacterium tuberculosis infection is endemic [8]. It is well known that DM impairs the immunity of patients and therefore is an independent risk factor for infections such as TB . The combination of DM and tuberculosis (TB) was life threatening in the pre-antibiotic era and before the readily available treatment of both diseases between DM and TB is re-emerging because the epidemiology of both diseases is progressive worldwide and cases of DM are increasing in the developing countries where TB is of high burden [9]. This work aims to illustrate the possible role of CMV and DM in the induction of immune anergy in TB patients. The marker of cell-mediated immunity and activated T cells will be the level of IFN-ɣ and IL-2.

2. MATERIALS AND METHODS

2.1 Patients and Controls

A total of 70 patients suffering from tuberculosis were involved in this case control study. Twenty nine of them were diabetic. They were admitted to the Specialized Chest and Respiratory Center in Baghdad, Iraq during the period October 2012 to January 2013.The

TB patients were diagnosed according to the clinical and laboratory criteria. A total of 30 apparently healthy individuals were involved as controls group. The age range of controls was approximately matched to the patients.

Three ml of blood were collected by vein puncture into sterile gel and clot activator vacuum tube, left for 30 minutes, then centrifuged at 3000 rpm for one minute. The serum was collected in a clean test tube and stored at – 20 °C until using it in serological tests [10].

2.2 Estimation of Anti-CMV IgG Antibody

The concentration of anti-CMV IgG antibody was estimated in the sera of patients and controls by enzyme linked immunosorbent assay-ELISA according to the instruction of manufacturer manual(Human –Germany).Antibodies class IgG was used here depending on the fact that IgG antibodies elevates and persists for long time as an indicator for chronic or previous infection with Cytomegalovirus.

2.3 Estimation of Interferon-gamma (IFN-ɣ) and Interleukin 2(IL-2)

The IFN- ɣ and IL-2 were measured in the sera of patients and controls by ELISA technique according to the instruction of manufacturer manual (Cusabio-China,and Abcam-USA)respectively. These cytokines were measured as a markers for Th1 response which is responsible mainly for cell-mediated immunity against TB.

2.4 Ethical Approval

The necessary ethical approval from the Specialized Chest and Respiratory Center in Baghdad-Iraq was obtained. Moreover, all subjects involved in this work were informed and the agreement required for doing the experiments and publication this work was obtained from each one prior the collection of samples.

2.5 Statistical Analysis

The t-test was carried out using a software program (SPSS 19.0,SPSS Inc.,Chicago,USA) to check the significance differences among the studied groups and p value less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

The mean of titer of Anti-Cytomegalovirus IgG in TB patients sera was 2.03 pg/ml, while in control subjects was 1.43 pg/ml (Table 1). This study shows a significant difference between TB patients and controls when p value was less than 0.001. This finding were matched with Karalian [11] who mentioned that infection of chronic Cytomegalovirus in patients with tuberculosis is rather significant and in several times exceeds similar parameters in healthy persons. Chronic viral infections have been identified as a risk factor for developing and complicated tuberculosis [12]. This finding also supports suggestion that cytomegalovirus activates tuberculosis from latent to active form. This is based on several epidemiological reports, and the plausible mechanism is the suppression of delayed type hypersensitivity after CMV infection [13]. Thus, depending on the results of this work, CMV infection lead to immune suppression causing decrease in the CMI while increased susptibility to TB infection during immune suppression.

In this study, the concentrations of IFN-γ and IL-2 were (0.195 pg/ml) and (0.409 pg/ml) respectively. There was no significantly difference for patients when compared to controls, (Table 1).

Table 1. Level of Anti-Cytomegalovirus IgG and Concentration of IFN-γ and IL-2

Subject	Anti-CMV IgG Mean(pg/ml) ±SD	IFN-γ Mean (pg/ml) ±SD	IL-2 Mean(pg/ml)±SD
Patients (n = 70)	2.03±0.46	0.195±0.034	0.409±0.145
Controls (n = 30)	1.44±0.46	0.190±0.043	0.470±0.146

The whole mark of normal immune response in TB patients is elevation on the level of Th1 cell cytokines mainly IFN-γ and IL-2 to an amount that much greater and significant than normal subjects. Whereas in this study there is no significant difference between two groups. This means that TB patients involved in this study have a low level of IL-2 and IFN-γ that may be insufficient for activation of T cell proliferation and activation of the mycobacteriocidal activity of alveolar macrophages.The results of non increasing TH1 cytokine profile in TB patients indicates the presence of reduced cell mediated in TB patients in spite of the presence of mycobacterial antigens. This unresponsiveness reflects an inhibitory signals for cell- mediated immunity induced by CMV which is considered as on viruses with potential activity to induce immune anergy. This finding was matched with Frascaroli et al. [14] who mentioned that CMV suppresses lymphocyte proliferation to T cell mitogens and prevents lymphocytes and monocytes from producing and responding to immune mediators, such as IL-1 and IL-2, in addition, CMV inhibits cytotoxic and NK cell activity [14]. Carsillo et al. [15] mention that there is *in vivo* and *in vitro* evidence of Th2 polarization in cytokine responses during and after CMV infection: production of IL-4 increases while the production of IL-2 and IFN-γ decreased [15]. Yu et al. [16] fund that CMV causes up-regulation of CD4+ and CD25+ Treg that contribute to the increased IL-10 and found significantly increased sustained IL-10 secretion. Wiertz et al. [17] reported that Cytomegaloviruses, as do many Herpes viruses, impair T cell activation by interfering with both MHC class I and II antigen processing and presentation. Cytomegalovirus is able to affect the capacity of infected cells to present antigen to T cells by interfering with the major histocompatibility complex-presentation pathway and induce a down-regulation of cellular proliferation [18].

This study shows a significant difference in the concentration of IFN-γ and IL-2 between diabetic and non-diabetic TB patients when the p value of both IFN-γ and IL-2 were less than 0.05, (Table 2).

Table 2. Effect of Diabetes Mellitus on Cytokine Profile in TB Patients

Subject	Diabetic (n = 29)	Non-diabetic (n = 41)	P value
IFN-γ (Mean ±SD)	0.184±0.033	0.202±0.032	0.024
IL-2 (Mean ±SD)	0.362±0.032	0.443±0.182	0.008

These results show a significant decrease in the concentration of both cytokines IFN-γ and IL-2 in diabetic TB patients. This finding was matched with Velez and Bhalla, (2012) reported that diabetic TB patients have lower serum IFN-γ and IL-2 concentration than non-diabetic TB patients [19]. Our finding is in agree with Syed et al. [20] who found that lower levels of serum IL-2, IL-6 and IL-10 in diabetic TB patients and explained that the presence of high levels of circulating glucose induce tolerance and lead to high resting cytokine production. Recent studies proofed that diabetic TB patients have an impaired ability to up-regulate

important cytokines and adhesion molecules, and these findings may provide a mechanistic explanation for the increased risk of and adverse outcome from infection that is observed in diabetic patients [21,22].

4. CONCLUSION

Thus from the results expressed above, CMV and DM may be considered as predisposing factors for TB by suppression the cell–mediated immunity and increasing the host susceptibility to tubercle bacilli.

CONSENT

All authors declare that written informed consent was obtained from the patient for publication of this research article.

ACKNOWLEDGMENT

Authors would like to introduce great thanks to all the patients, and healthy subjects involved in this study. Thanks also for all the staff of Specialized Chest and Respiratory Center in Baghdad, Iraq.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. WHO. Global tuberculosis control: epidemiology, strategy, financing. Geneva. 2012;6-33.
2. Gagneux, S. Host – pathogen coevolution in human tuberculosis. Phil. Trans. R. Soc. B. 2012;367:850–859.
3. Shiloh MU, Champion PA. To catch a killer: What can mycobacterial models teach us about *Mycobacterium tuberculosis* pathogenesis? Current Opinion in Microbiology. 2010;13(1):86–92.
4. Matzinger P. The Danger Model: A Renewed Sense of Self. Science. 2002;296:301-305.
5. Mishahidi, S.; Huang, CT. and Sadegh-nasseri, S. Anergy in peripheral memory CD4+ T cells induced by low avidity engagement of a T cell receptor. Journal of Experimental Medicine. 2001;194:719-731.
6. Crough, T. and Khanna, R. Immunobiology of human cytomegalovirus: from bench to bedside. Clin. Microbiol. Rev. 2009;22:76-98.
7. Arnold, CR.; Wolf, J.; Brunner, S.; Herndler-Brandstetter, D. and GrubeckLoebenstein, B. Gain and loss of T cell subsets in old age-age-related reshaping of the T cell repertoire. J. Clin. Immunol. 2011;31:137-146.
8. Ruder, K. Fighting the epidemic: a United Nations resolution on diabetes. Diabetic Forecast. 2007;60:50-51.
9. Dixon, B. Diabetes and tuberculosis: an unhealthy partnership. Lancet Infect. Dis. 2007;7:444.
10. Lewis, S. M.; Bain, B. J. and Bates, I. Dacie and Lewis Practical hematology. 9th ed. London: Chrchill Livingston; 2001.

11. Karalian, MA. Chronic virus infections in combination with tubercular process. Georgian Med. News. 2008;159:40-43.
12. Wang, Ying-Chuan.; Chiu, Chun-Hsiang; Lin, Yu-Cheih; Shen, Hueng-yuan; Yang, Ya-Sung and Wang, Ning-Chi. Fever in a Patient with Liver Cirrhosis: Tuberculous Peritonitis with Cytomegalovirus Co-infection. J. Med. Sci. 2012;32(5):239-242.
13. Lee, AW.; Hertel, L.; Louie, RK.; Burster, T.; Lacaille, V.; Pashine, A.; Abate, DA.; Mocarski, ES. and Mellins, ED. Human cytomegalovirus alters localization of MHC class II and dendrite morphology in mature Langerhans cells. J. Immunol. 2012;177:3960-3971.
14. Frascaroli, G.; Varani, S.; Moepps, B.; Sinzger, C.; Landini, MP. and Mertens, T. (). Human cytomegalovirus subverts the functions of monocytes, impairing chemokine-mediated migration and leukocyte recruitment. J. Virol. 2011;80:7578-7589.
15. Carsillo, M.; Kay, K. and Stefan, N. Cytokine Imbalance after cytomegalovirus infection. J. Virol. 2009;83(14):7244-7251.
16. Yu, XL.; Yu-Ming, C.; Bi-Sheng, S.; Famg-Zeng, Q.; Feng-bin, W. and Xi-nian, L. Cytomegalovirus infection in adults induce production of IL-10 and is associated with increased CD4+CD25+ regulatory T cells. J. Immunol. 2008;181:7356-7366.
17. Wiertz, K.; Barzilai, O.; Sherer, Y.; Ram, M.; Izhaky, D.; Anaya, JM. and Shoenfeld, Y. Epstein-Barr virus and cytomegalovirus in autoimmune diseases: are they truly notorious? A preliminary report. Ann. N. Y. Acad. Sci. 2008;1108:567-577.
18. Brunner, S.: Herndler-Brandstetter, D.; Weinberger, B. and Grubeck-Loebenstein, B. Persistent viral infection and immune aging. Immunity and Aging. 2011;10:362-369.
19. Velez, Maria-Gabriela and Bhalla, Vivek. The Role of the Immune System in the Pathogenesis of Diabetic Nephropathy. J. Nephrol. Therapeutic. 2012;423:1231-39.
20. Syed, Sulaiman; Mohd, Zain; Abdul Majid, S.; Munyin, N.; Mohd, Tajuddin; Khairuddin, Z. and Ban, Hing. Tuberculosis Among Diabetic Patient. WebmedCentral Infectious Diseases. 2011;2(12):1690-1703.
21. Dooley KE, Chaisson RE. Tuberculosis and diabetes mellitus: convergence of two epidemics. Lancet Infect. Dis. 2013;9:737-746.
22. Wang CS, Yang CJ, Chen HC, Chuang SH, Chong IW, Hwang JJ, Huang MS. Impact of type 2 diabetes on manifestations and treatment outcome of pulmonary tuberculosis. Epidemiol. Infect. 2013;137:203-210.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=306&id=12&aid=2338