INTERACTION OF LATENT EPSTEIN-BARR VIRUS AND CYTOMEGALOVIRUS REACTIVATION WITH PAIN LEVELS BETWEEN TWO DIFFERENT AGE GROUPS

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Abstract - Inflammageing, immunosenescence and aging altogether serve as initiator for pain level by increasing the opportunity of herpesviruses reactivation by (EBV and CMV) after latent period, because of impaired immunity. So, the current study assumed to study the potential interaction between these viruses and pain levels, which had been evaluated by interleukins (ILs-6, 7) and CRP estimation in two different age groups. Serum samples of two age groups (100 participants) as young ages (ranged: 20 – 35) and old ages (≥ 50) years, were examined for antibody titers of (EBV VCA and CMV IgGs), interleukins (ILs-6, 7) and CRP levels by the method of Enzyme Linked Immunosorbent Assay (ELISA). Besides, pain (SF-36 pain subscale) was assessed between these groups. Interestingly, the old ages (≥ 50 years) were with a strong significant positive association ($p<0.001$; $r = 0.689$) with higher pain levels (65.402±13.566) and high titer of serum EBV VCA-IgG (2.1816±0.558), followed by week positive correlation with CMV-IgG as a latent herpesviruses reactivation. Furthermore, multiple linear regression test suggested that higher pain levels were associated with variation in age groups ($p<0.001$), EBV, CMV and IL-6 ($p<0.05$) among individuals with higher pain but not CRP or IL-7. it was concluded, higher pain level was estimated among the old ages of participants and it was consequently related with EBV and CMV reactivation and up-regulation of IL-6.

Keywords - CRP, CMV, EBV, IL-6, IL-7, PAIN.

I. INTRODUCTION

Epstein-Barr Virus (EBV) and Cytomegalovirus (CMV) are herpesviruses and bout 95% of the adult population worldwide being seropositive for them in the first years of life as asymptomatic infection. Both stays as a latent after primary infection [1,2]. After the weaken of cellular and adaptive immunity at aging people a cycle of reactivation and replication of the latent EBV and CMV activate to produce high titers of IgG as a biomarker of immune dysregulation [3]. This is one of the trigger factor of immune system aging which had been explored in many studies by correlations of EBV and CMV infection with biomarkers of declining immune system. A persistent herpesvirus infection effects immunosenescence because of aging; when pro-inflammatory and anti-inflammatory cytokines \ chemokines mechanisms imbalanced which is defined as "inflammaging" [4]. Tt consequently effects on differences in cytokine production and releasing levels. Now, it seems to be the EBV and CMV act as a chronic co-factorsfor cytokine action with importanteffect on the aging of immune system and thus acts as a modulating factors of inflammaging status and both has been linked to increased multiple chronic diseases in older adults. Moreover, it has been shown the body pain after the 60 years of age as a twofold level compared to young ages and related with decreasing of life quality or daily stress level through living time or influences on a set of (molecular, cellular, physiological, immunological and psycho-social events) [5]. Human aging is possibly under the control of intrinsic (genetic factors) like; metabolism, catabolism functions, and extrinsic (environmental factors) such as; EBV and CMV infections [6]. furthermore, recent studies support the hypothesis for IL-7 in the maintenance of a vital health span and have identified "the IL-7 network," as a beneficial biomarker of successful aging [7]. Chronic raise of CRP in the healthy aging people may related with multiple ageing-related chronic diseases. Some studies suggest the IL-6 up-regulation and elevated CRP level are considered among the most commonly indicators of inflammation process and other not [8] Finally, immunosenescence is a fundamental part of human aging that lead to chronic low-grade inflammation (inflammaging) which is a decrease in cellular immunity, defective antibodies and autoantibodies production [4]. Chronic viral infections and elevations of proinflammatory cytokines concentrations are triggers of inflammaging [9]. Furthermore, pro-inflammatory cytokines stimulate the C-reactive protein (CRP) synthesis in the liver. So,this study aimed to investigative the association between pain levels, latent EBV and CMV reactivation and markers of inflammation in two different age groups (old and young).

II. PARTICIPANTS

One hundred serum samples from both sexes of participants (Male=54, Female=46), aged (20 - 65 years) were enrolled in a cross-sectional study who had a normal life-style without chronic diseases, auto-immune diseases, cancers or any other inflammatory disorders and non-pregnant women from different

medical staff workers of sheikh Zayed Hospital \ Baghdad, which were selected from January to the 30th March 2019. Furthermore, participants were divided into two groups as a young ages (20-35) and old ages (≥50). Study protocol was reviewed and approved by the local ethics committee.

III. MATERIALS AND METHODS

Serum samples in the current study were submitted into Enzyme Linked Immunosorbent Assay (ELISA) procedure to analyze the studied biomarkers by following the instruction manuals for each company as labeled in table (1) below. Participants completely answered on a self-administrated questionnaire. In addition, anthropometric measurement, including BMI was done.Furthermore, pain level determined by using, The RAND 36-Item Short Form Health Survey (SF-36): which consist of eight scales: 1) Physical functioning, 2) Role limitations due to physical health, 3) Role limitations due to emotional problems, 4) Energy/fatigue, 5) Emotional well-being 6) Social functioning, 7) Pain, 8) Role emotion, and 8) General health. The SF-36's bodily pain subscale form was used to evaluate pain severity levels via two objects ('How much bodily pain have you had during the entire past 4 weeks?'; 'Throughout the past 4 weeks, how much did pain interfere with your normal work activity (including both work outside and housework?'). The answer scale ranges from 0 to 100, with higher scores representing less severe, lower impact pain.

Table (1): Enzyme linked immunosorbent assay (ELISA) kits for detection of (viral-IgGs, cytokine levels and CRP) in patients sera.

Biomarkers	Supplier	Sensitivity: Detection range
Epstein-Barrvirus (EBV VCA-IgG)	Abnova-GmbH, Germany	>1.1
Cytomegalovirus (CMV-IgG)	Diagnostic Automation \ Cortez diagnostic Inc., USA	> 1.2 IU/ml
Interleukin-6(IL-6)	Invitrogen., USA	<2 pg/mL: 7.8-2500 pg/mL
Interleukin-7(IL-7)	Invitrogen., USA	45 pg/mL: 4.1-1000 pg/mL
C-Reactive protein (CRP)	MyBioSource Inc., USA	0.23ng/mL: 0.39-25ng/mL

IV. STATISTICS

The IBM, SPSS statistics software 25.0 version was used to analyze the collected data of the current study. The (*p.value*<0.05) were considered statistically significant.

- Descriptive data analysis: Frequency analysis (Table 1).
- Independent *t*-Test:for means comparison (Table 2).
- Pearson Correlation:to examine relation between variables (Table3).
- Multiple regression analysis: to test our study hypothesis by predicting which one of the studied biomarkers effect on the pain level (Table 4).

V. RESULTS

Table (1): Baseline data and biomarkers of study population characteristics

Baseline Data \ Biomarkers		No. (%)	Mean±SD (Range)
Age Groups	Young	44 (%)	43.26±15.22 (20 – 65)
	Old	56 (%)	
Genders	Male	54 (%)	-
	Female	46 (%)	-
Marital status	Married	76 (%)	-
	Unmarried	24 (%)	-
BMI Scale	Normal weight	73 (%)	25.45±8.04 (18 – 46)
	Overweight	27 (%)	
Smoking status	Smoker	62 (%)	-
	Non-smoker	38 (%)	
SF-36 Pain (reversed)	-	-	47.90±25.40 (0 – 95)
EBV-VCA IgG	Positive	65 (%)	1.71±0.70 (0.20 – 2.83)
	Negative	35 (%)	
CMV-IgG	Positive	22 (%)	1.07±0.84 (0.06 – 2.90)
	Negative	78 (%)	
IL-6	-	-	17.40±11.06 (3.98 – 39.70)
IL-7	-	-	16.73±11.28 (3.72 – 41.32)
CRP	-	-	20.13±6.50 (10.02 – 33.98)

SD = Standard Deviation, BMI= Body Mass Index, CRP = C-reactive protein.
EBV-VCA IgG = Epstein Barr Virus, viral capsid antigen Immunoglobulin-G.
CMV-IgG = Cytomegalovirus Immunoglobulin-G.
IL-6, IL-7 = Interleukins 6, 7.
SF-36 Pain = RAND 36-Item short form health survey pain subscale. **Note:** SF-36 pain subscale was reversed, for easier result interpretation, lower SF-36 pain values reflect lower pain levels, and higher values reflect higher pain levels.

Table (1) summarized the demographic characteristics of all participants, 56 (56%) of old ages and 44 (44%) of young individualswitha pain level (ranged: 0-95). In addition to, 65 (65%), 22 (22%) of them had been with a positive EBV VCA-IgG and CMV-IgG respectively. While, most of participants were males 54 (54%), married 76 (76%) with normal BMI 73 (73%) and 62 (62%) of them were a smoker volunteers. Furthermore, interleukins (IL-6 and Il-7) with CRP levels showed a graduated results from low to high concentration regarding to the selected age groups as explained in table 2.

Table (2): Comparison association of the mean levels among studied biomarkers and baseline data according to the study groups

Baseline Data Biomarkers	Study Groups (n=100) Mean±SD		t. Test	*p.value*
	Young (n=44)	Old (n=56)		
Age	26.89±4.933	56.13±3.876	-33.203	<0.001
BMI	24.18±7.583	26.45±8.318	-1.404	0.16
IL-6	10.8523 ± 6.212	22.5482 ± 11.355	-6.144	<0.001
IL-7	12.2925 ± 8.272	20.2291 ± 12.140	-3.710	<0.001
CRP	16.3245±4.368	23.1314 ± 6.357	-6.063	<0.001
EBV-VCA IgG	1.1175±0.302	2.1816±0.558	-11.383	<0.001
CMV-IgG	0.7191±0.648	1.3609±0.881	-4.043	<0.001
SF-36 Pain (reversed)	25.625±18.537	65.402±13.566	-12.387	<0.001

Independent *t*-Test was applied to test the mean comparison of biomarkers and baseline data according to study groups,data were presented as mean ± SD.
Note: SF-36 pain subscale was reversed, for easier result interpretation, lower SF-36 pain values reflect lower pain levels, and higher values reflect higher pain levels.

Table (2) demonstrate the comparison association of the mean levels between study groups, it was with the high significant difference among all demographic characteristics (P < 0.001) except for BMI (*P* = 0.16).

The correlation between SF-36 pain subscale with studied variables exposed high significantly positive associations between them, but there was no association with BMI and CMV-IgG seropositivity as represented in table (3).

Table (3): Correlation of SF-36 pain subscale with other studied variables

Variables	Pearson Correlation (r)	*p.value*
Age	0.781	<0.001
BMI	0.121	0.116
IL6	0.514	<0.001
IL7	0.326	<0.001
CRP	0.474	<0.001
EBV-VCA IgG	0.689	<0.001
CMV-IgG	0.150	0.068

Pearson Correlation test was applied to test the correlation of SF-36 pain subscale with biomarkers and baseline characteristics

Table (4): Multiple regression analysis for variables predicting SF-36 pain subscale with estimated coefficient (B, SE), t and *p.value*.

Variables	B	SE	t	*p.value*
age	0.985	0.159	6.188	<0.001
BMI	-0.247	0.200	-1.235	0.220
IL6	0.718	0.274	2.619	<0.05
IL7	-0.444	0.236	-1.882	0.063
CRP	0.024	0.289	0.083	0.934
EBV-VCA IgG	8.459	3.362	2.516	<0.05
CMV-IgG	-5.445	1.912	-2.848	<0.05

SE= Standard Error

Regression analysis were included (viral –IgGs, cytokines and baseline data) had been predicted that the age categories, IL-6, EBV VCA-IgG andCMV-IgG was the only significant and effective variables regarding to the pain subscale levels comparing to otherstudied variables as in table (4).

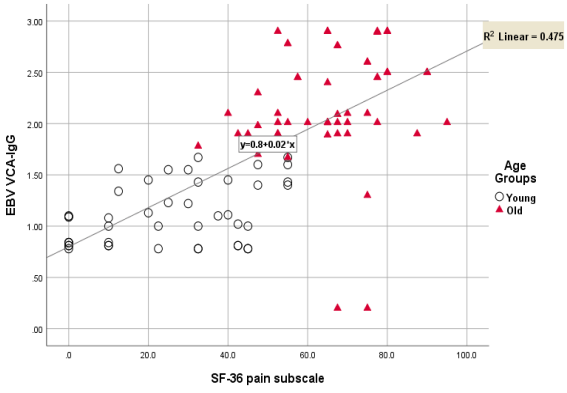


Figure 1: Scatter diagram represented the correlation between EBV VCA-IgG with pain levels according to the age categories of the study groups.

It was a strong and positive significant association between the EBV VCA antibody titers activation and pain subscale regarding to the age groups.

Note: Higher SF-36 values indicate higher pain, lower SF-36 values indicate lower pain.

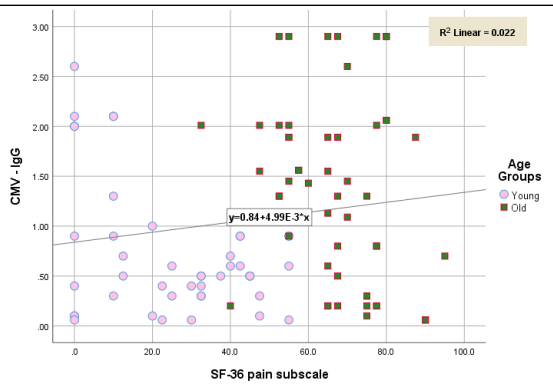


Figure 2: Scatter diagram represented the correlation between CMV-IgG with pain levels according to the age categories of the study groups.

It was a weak and positive significant association between the CMV antibody titers activation and pain subscale regarding to the age groups.

Note: Higher SF-36 values indicate higher pain, lower SF-36 values indicate lower pain.

V1. DISCUSSION

The recent Iraqi study was the first to report pain-associated anti-EBV and anti-CMV IgG titers as a latent reactivation in two different age groups (young and old), and it had been supposed the persistent viral infections in older may interfere significantly with immune dysregulation "immunosenescence" resulting impair cytokines secretion which called (Inflammageing) which are collectively related significantly with pain level. In this study, older age was associated with elevated EBV VCA and CMV IgGs antibody titers with higher pain levels (SF-36 pain subscale 65.4). Alteration of immune responses after the fiftieth have clinical impact which may led to "immunosenescence" diminished effectiveness of the immune system [6]. Thus, the relationship between older age and EBV VCA and CMV IgGs antibody titers among individuals who reported high pain level proposes that pain act as a chronic stressor with potential influences on immune dysfunction in older individuals. Our recent outcomes agree with previous study which indicating that several triggering factors are responsible for impairing the balance between latency and the reactivation of viral infection [10], and the chronic stress such as pain has shown to modify the HSV-specific immune response to latent infection [11]. In addition to, there's evidence from geriatric researches, recording cellular immune dysregulation in accordance with latent EBV in the older ages [12]. Furthermore, a study suggesting that latent herpes viruses reactivation can induce inflammation [13], and significant relationship between pain with latent (EBV, CMV) reactivation and inflammation in the current sample, which is agree with a research that shown a greater herpes virus burden is related with inflammation [14,15]. Particularly, seropositivity for both EBV and CMV has been found to be significantly related with high levels of CRP and IL-6 [9]. A recent study shows significant association between latent herpesvirus reactivation and inflammation in larger sample size [16]. The pathogenesis of aging-associated inflammation, resulting from many of co-factors like; environmental, viral infections, and genetic factors, is not well studied but ageing-associated IL-6increaseshad been frequently measured [17,18].

CRP is acute-phase protein which is triggered by IL-6 then inducing TNFα and IL-1β expression [8], and in the same time, CRP may act as a protection factor against different inflammatory diseases by interfering with several mediators like; IL-10 and IL-12 [19]. Moreover, asignificantly serological study observed elevated levels of CRP, IL-6 and IL-13 in healthy ageing people [8],different suggestionstelling a possible linking of latent/persistent viral/ infections such as herpesvirus infection as a part of process of (Inflammageing) which may finally induce immune imbalance or disturbances during healthy ageing intervals [4,8,19]. As example, CMV can induce inflammatory cytokines production and may trigger a variety of age-associated inflammatory diseases, because it infected mononuclear cells to produce a diversity of cytokines such as IL-2, IL-6 and TNF-α [20]. Additionally, the age considers as a risk factor for different diseases in studies done in Holland indicate that IL-7 and IL-7R may provide hopefulimmunosenescencebiomarkers as a low (IL-7R) expression in olderages was linked with a healthier metabolic process (e.g., insulin sensitivity and lower lipids) and thus chosen as a friendly biomarker for healthy aging detection [21,22]. But this does not suggest that low IL-7R expression generally is useful to preserving a better health status. Concept of low IL-7 receptor expression are useful for attainmenta healthy old age in comparison of patients with different inflammatory diseases andhigh levels of the genes of IL-7R /ligand complex unit [23], and that the IL-7 unit or the IL-7 receptor may givea conceivable treatments [24]. So, in aging population in which they have lower IL-7 receptorlevel lived much longer because it enough to preserve peripheral T cells to preserve immune system function and transient intonation of IL-7 receptor is one theoreticallyactive strategy to scope this goal. Finally, a longitudinal study to explore the immune dysfunction in older individuals with latent viral infection is needed, additional markers of immune dysregulation (e.g., IL-1β, IL-6, Natural Killer (NK) cells) to provide a more comprehensive understanding of how pain may adversely affect immune function in seniors and, a large sample size should be used. Despite, many reports explored the association of herpesviruses infection with heart diseases, blood pressure,allergic diseases, autoimmune diseases or even cancers had been carried out, but a few researches have been done related older adults having chronic pain with

persistent viral infection. Therefore, further studies are needed.

VII. CONCLUSIONS

It was concluded the latent EBV and CMV reactivation with over secretion of IL-6 is related with older age people who reported higher pain levels.

RECOMMENDATIONS

A molecular study of herpes virus reactivation and its interferences role with interleukins up-regulation in older people is needed for the prediction of a new disease susceptibility strategies, target therapies and preventing many chronic age-related disease. Further studies are needed to clarify the reliability of these outcomes.

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Conflicts of interest: Nil

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