Study the possible Seropositivity connection of EBV, Rubella Virus & CMV infection with four groups of autoimmune diseases in sample of Iraqi patients

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Abstract

Autoimmune diseases are complex and happens when the human immune system attacks tissue host for unknown etiology or maybe by some other genetic cofactors or environmental agents (mainly viral particles, bacterial and other infectious agents; by acting as a major cofactors and serving as important initiators in the disease progression for a specific autoimmune sickness and not causing illness on their own) or even as complications or opportunistic infections associated with the disease. The present study aimed to: (I) study the linking of certain infectious viral agents with human autoimmune diseases. (II) Determine possible roles of some studied clinico-pathological factors like; gender, age and to investigate the prevalence of Seropositivity of the antibodies titer of EBV, Rubella virus and CMV with autoimmune diseases development in human. In this study, four groups candidates of Celiac diseases (CD) n=31, Diabetes Mellitus –I (DM-I) n=30, Rheumatoid Arthritis (RA) n=30 & Systemic lupus erythematous (SLE) n=30 were studied from 1^{st} – 30^{th} of January 2017 at Medical City \ Baghdad. Demographic and laboratory data were collected. All the samples were examined for the presence of EBV VCA-IgG, Rubella IgG and CMV IgM by Indirect and Sandwich ELISA techniques by using a commercial kits from DIAGNOSTIC AUTOMATIONS CO. \ USA. A total of 121 autoimmune disease patients were studied , the overall positive results for EBV VCA IgG was (68.6 %) while for Rubella IgG was (50.4%) and for CMV IgG was (52.1%) with significant differences in comparing with the negative results ($p < 0.05$).

Celiac disease and diabetic type-I patients had been detected with high Seropositivity for EBV VCA IgG as (22.3%) for each of them, While the high positive results of Rubella IgG was detected in Celiac disease patients as (16.5%). Finally, the diabetic Type-I patients showed the most high Seropositivity for CMV IgG as (17.4%) in significant differences with the negative results regarding to all the other positive ones ($p < 0.05$).

Keywords: EBV, Rubella virus, CMV, Autoimmune diseases.

دراسة إمكانية ارتباط الايجابية المصلية للاصابة بفيروس ابشتاين بار، فيروس الحصبة الالمانية والفيروس المضخم للخلايا مع أربعة مجاميع من إمراض المناعة الذاتية في عينة من المرضى العراقيين

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الملخص

أمراض المناعة الذاتية معقدة وتحدث عندما يهاجم جهاز المناعة أنسجة المضيف لسبب غير معروف أو ربما لبعض العوامل الوراثية الاخرى أو مؤثرات بيئية (خاصة الفيروسات والبكتيريا والكائنات الحية الدقيقة المعدية الاخرى ؛ وذلك بالقيام بدور العوامل المساعدة الرئيسية والتي تعمل كمحفز هام في تطور المرض إلى مرض محدد من إمراض المناعة الذاتية ولا تسبب المرض بنفسها) أو حتى من الممكن إن تكون مصاحبة للمضاعفات أو االلتهابات الانتهازية للمرض. هدفت الدراسة إلى: (I) مناقشة الادلة التي تربط بعض العوامل المعدية (الفيروسات) مع أمراض المناعة الذاتية في البشر. (II) تحديد الادوار المحتملة لبعض العوامل المرضية الاكلينيكية المدروسة مثلا ؛ الجنس والعمر والتحري عن انتشار إيجابية المصل لمعيارية االجسام المضادة لفيروس ابشتاين بار، فيروس الحصبة الالمانية والفيروس المضخم للخلايا مع تطور أمراض المناعة الذاتية في البشر. في هذه الدراسة ، أربعة مجاميع مشاركة من الداء الزالقي \ عدد = 31 ، مرض السكري من النوع الاول \ عدد=31 ، التهاب المفاصل الرثوي \ عدد = 30 و الذئبة الحمراء \ عدد = 30 ، درست من تاريخ (1 – 30 يناير2017) في مدينة الطب \ بغداد. وقد تم جمع البيانات الديموغرافية و المختبرية. وفحصت جميع العينات لوجود EBV VCA-IgG، Rubella virus IgG ، CMV IgM بواسطة تقنية ELISA الـ الغير المباشرة والساندويش باستخدام معدات مختبريه تجارية من DIAGNOSTIC. AUTOMATIONS CO. \ USAمن مجموع 121 من مرضى المناعة الذاتية التي تم دراستها ، كانت النتائج االيجابية الشاملة لـ EBV VCA IgG(68.6%)، في حين Rubella virدIgG us كانت (50.4٪) و CMV IgM كانت (52.1٪) مع وجود اختالفات كبيرة مقارنتا مع النتائج السلبية (p>0.05) . تم الكشف عن الداء الزلاقي وداء السكري من النوع الاول باستجابة مصلية عالية لـ EBV VCA-IgG كـ (22.3%) لكل منهما، في حين أن النتائج ألايجابية العالية من Rubella virus IgG تم الكشف عنها في المرضى الذين يعانون من مرض الداء الزالقي كنسبة (16.5%). وأخيرا، أظهر المرضى بداء السكري من النوع الاول استجابة مصلية عالية لـCMV IgM كنسبة (17.4%) وفي اختالفات كبيرة مع النتائج السلبية وعلاقتها بالنتائج الايـــــجابية الاخرى (p < 0.05).

Introduction

Autoimmune disease and virus infections have long been connected. These infections often follow the incidence of inflammation in the target organ. Numerous mechanisms often used to clarify the relationship of autoimmunity and viral infection are bystander activation, molecular mimicry, and viral persistence [1]. Many reviews connected of Epstein-Barr virus (EBV) and Rubella virus or Cytomegalovirus (CMV) with systemic autoimmune diseases SAD development. Many immune

modulating functions make EBV or some infectious agents a good candidate for initiation of autoimmune disorders and disease progression. This assessment focuses on Celiac diseases, Diabetes Mellitus –I (DM-I), rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [2, 3].

The chronic infections are significant in a diversity of autoimmune disorders and neurodegenerative diseases processes. It beforehand suggested that many and perhaps a majority of these Systemic chronic infections (caused by viral agent like; CMV, HHV6, EBV or enterovirus, etc. or bacteria such as Mycoplasma, Chlamydia, etc.) in a process called apoptosis can directly kill or damaging the nerve cells, then nervous system degeneration was resulting, by that can stimulate an autoimmune reaction [4].

This can happen by diverse mechanisms. The most one mechanism that has attracted us is that when certain pathogen, such as some species of mycoplasmas, departure from invaded cells, they transmit part of the cell membrane of the host on their surface. This may activate the immune system to react to the host antigens on the foreign pathogen or certain microorganisms. Otherwise, certain microorganisms show surface antigens that mimic host cell surface antigens, and these may motivate autoimmune reactions [4, 5].

In addition to, there are several tools used by a certain pathogen can cause autoimmunity progression in the host. The infectious agent may have components that are analogous enough in structure to self-antigen or amino acid sequence that the pathogen agent actions as a self-‘mimicry’. Named ‘molecular mimicry’, T-cell or B-cells that are triggered in pathogen response are also cross - reactive to self and main to direct damage and of the immune system as additional activation of other immunity arms [6].

The antigen presenting cells (APCs) are taken up the antigens released from damaged tissue, and this produce a self-specific immune responding. The ‘Bystander activation’ explain indirect activation of autoimmune cells or a non-specific response caused by the inflammatory environment existing during infection period. Finally, infection may chief autoimmunity disorders by the processing and performance of the ‘cryptic - antigens’. In difference to dominant of antigenic determinants, and the subdominant cryptic- antigens are normally unnoticeable to the immune system. [7].

Materials and Methods

The Diagnostic Automation Inc. of Sandwich EBV-VCA-IgG test and Indirect Rubella IgG , CMV- IgG \ ELISA tests system is deliberate to detect IgG-class Abs to these viruses in the sample of human-sera [8]. Plastic microwell strips were prepared by the passive absorption ways with viruses-coated antigen. The test process involved: -

1. The sera (diluted) were incubated in Ag-coated plate. And then any Ags-Abs specific in the sample will bind to the coated-antigen. The microtiter-plate was washed to eliminate boundless Abs and other components of serum.
2. The Peroxidase-conjugated goat of anti-human IgG was added to the wells and the plate was incubated. The IgG antibody immobilized on the solid phase in step 1 with react with the Conjugate. The wells were washed-off to remove the unreacted conjugate.

3. The microwells of plate holding the Immobilized-Peroxidase. Incubation of the conjugate with Peroxidase Substrate Solution. Then produces a color alteration by hydrolysis of the Substrate by Peroxidase. After that, the reaction was entirely stopped and the color intensity of the solution was measured photometrically. The intensity of color of solution measured by the Abs concentration in the test sample.

3. Statistical analyses

All statistical analyses were performed using SPSS ver. 18.0 and Excel application (2010). The association between the studied variables were analyzed using the Chi-square test, was used to detect the significances between variables of our study.

S= Significant difference ($P<0.05$). NS= Non Significant difference ($P>0.05$).HS = Highly Significant difference ($p<0.3301$) [9].

4. Results and Discussion

4.1. Results

According to the tables (1 and 2): In total 121 patients were studied, Thirty one (n=31\25.6%) was Celiac disease (CD) as a first group, the majority in this group were females (17 \14%) in ($\geq$ 45) of age interval as (16\13.2%) with high Seropositivity for EBV VCA-IgG as

(27\22.3%) followed by Rubella IgG which estimate as (20\16.5%) in significant differences (p< 0.05) regarding to other results in the CD patients group.

The Second group of thirty individuals (n=30\24.8%) were Diabetic type –I patients, with same genders percentages distribution as (15 \ 12.4%) for both genders and most of them were in (10-30) of age interval as (18 \ 14.9%) with high Seropositivity for EBV VCA-IgG as (27\22.3%) followed by CMV IgG as (21\17.4%) in significant differences with other data in DM-I patients group.

Rheumatoid Arthritis (RA) patients as third group was (n=30 \ 24.8%) individuals with high majority of female patients by (23\19%) in (10-30) of age interval as (14\11.6%) in high Seropositivity for EBV VCA-IgG as (23\19%) followed by Rubella IgG which estimate as (18 \14.9%) with significant differences (p< 0.05).

The last group was for systemic lupus erythematosus (SLE) patients as (n=30 \ 24.8%) most of them were females as (16 \13.2%) in (31-40) of age interval by (13 \ 10.7%) in high Seropositivity for Rubella IgG and CMV IgG which estimate as (7 \ 5.8%) to each one of them with significant differences regarding to the rest of results in SLE group (p< 0.05).

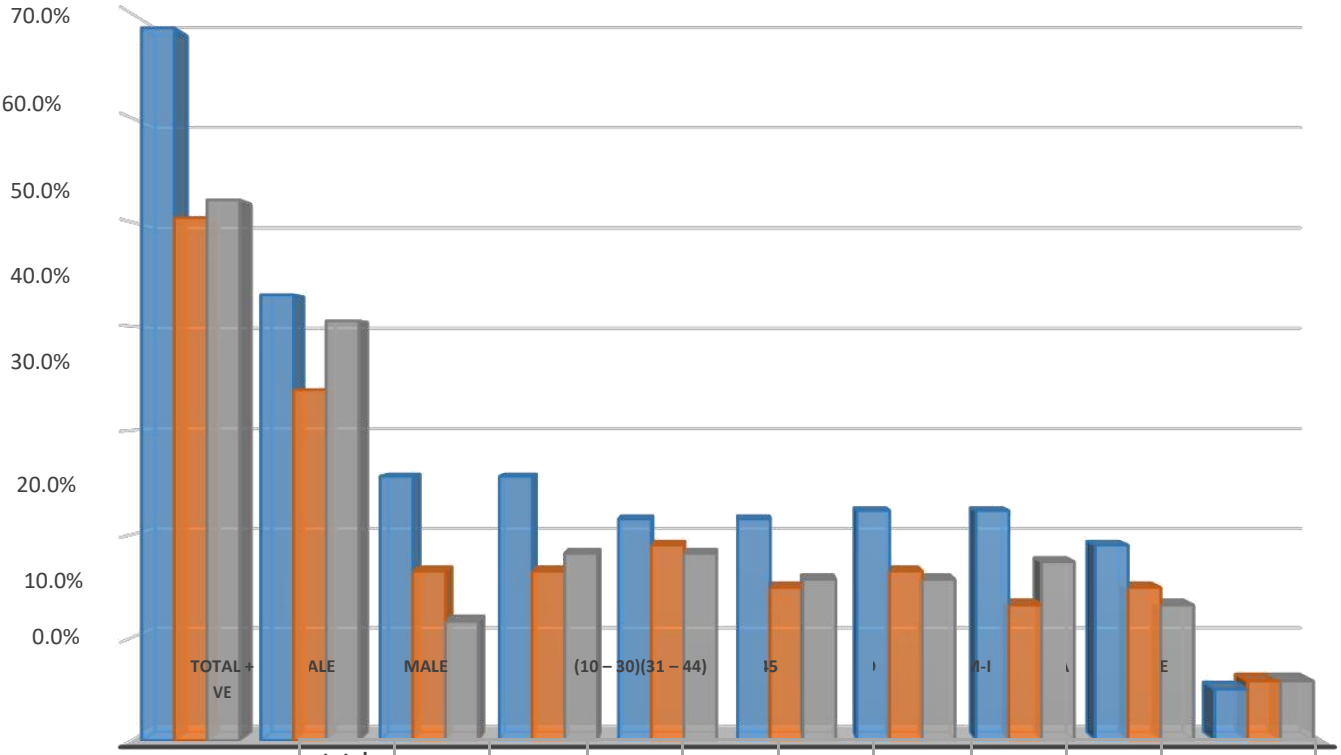
Table 1: Relationship of clinic-pathological factors with Types of auto-immune diseases

Clinic-pathological factors		Types of auto – immune diseases					p.value
		CD	DM – I	RA	SLE	Total	
Genders	Male	14 11.6%	15 121.4%	7 5.8%	14 11.6%	50 41.3%	0.1 NS
	Female	17 14%	15 12.4%	23 19%	16 13.2%	71 58.7%	
	Total	31 25%	30 24.8%	30 24.8%	30 24.8%	121 100%	
Age Groups	(10-30)	2 1.7%	18 14.9%	14 11.6%	6 5%	40 33.1%	0.000

	(31- 44)	13 10.7%	7 5.8%	7 5.8%	13 10.7%	40 33.1%	HS
	≥ 45	16 13.2%	5 4.1%	9 7.4%	11 9.1%	41 33.9%	
	Total	31 25.6%	30 24.8%	30 24.8%	30 24.8%	121 100%	

Table 2: Relationship of viruses with Types of auto –immune diseases

Viruses	**Results**	**Type of auto – immune diseases**					P.value
		CD	DM-I	RA	SLE	Total	
EBV VCA-IgG	**Positive**	27 22.3%	27 22.3%	23 19%	6 5%	83 68.6%	0.000 HS
	Negative	4 3.3%	3 2.5%	7 5.8%	24 19.8%	38 31.4%	
	Total	31 25.6%	30 24.8%	30 24.8%	30 24.8	121 100%	
Rubella IgG	**Positive**	20 16.5%	16 13.2%	18 14.9%	7 5.8%	61 50.4%	0.006 S
	Negative	11 9.1%	14 11.6%	12 9.9%	23 19%	60 49.6%	
	Total	31 25.6%	30 24.8%	30 24.8%	30 24.8%	121 100%	
CMV IgG	**Positive**	19 15.7%	21 17.4%	16 13.2%	7 5.8%	63 52.1%	0.002 S
	Negative	12 9.9%	9 7.4%	14 11.6%	23 19%	58 47.9%	
	Total	31 25.6%	30 24.8%	30 24.8%	30 24.8%	121 100%	



	total ve	Male	Female	(10 – 30)	(31 – 44)	≥ 45	CD	DM-I	RA	SLE
BV VCA - IgG	68.6%	43.0%	25.6%	25.6%	21.5%	21.5%	22.3%	22.3%	19.0%	5.0%
ubella IgG	50.4%	33.9%	16.5%	16.5%	19.0%	14.9%	16.5%	13.2%	14.9%	5.8%
MV IgM	52.1%	40.5%	11.6%	18.2%	18.2%	15.7%	15.7%	17.4%	13.2%	5.8%

Fig. 1: Total positive results for studied viruses According to the Types of auto - immune diseases and clinico-pathological factors

According to figure (1): That demonstrate the positive results for the studied viruses, the positivity of EBV VCA-IgG estimated as (83\68.6%) which has been detected in males by (52/43%) with high tendency to the (10-30) of age interval as (31\ 25.6%), particularly in CD and DM-I patients which representing by (27\22.3%) for each of them.

Regarding to the Seropositivity of Rubella IgG it was totally as (61\50.4%) which has been detected in males more than females as (41\33.9%) and most of them in (31-44) of age interval which represented as (32\19%), particularly in CD patients followed by RA patients as (20\16.5%) and (18\14.9%) respectively.

The CMV IgG Seropositivity estimated by (63\52.1%) which was distributed in males more than females as (49\40.5%) in (10-30) and (31-44) of age interval as (22\18.2%) for

each age period, most of them were with diabetes mellitus – I followed by Celiac disease as (21\17.4%) and (19\ 15.7%) respectively.

4.2. Discussion

The EBV had been doubted of participation in the process of the pathogenesis of numerous chronic autoimmune disorders meanwhile in 1971; the discovery of raised levels of antibody to the EBV in systemic lupus erythematosus (SLE) [3]. Normally EBV infection effect has been acknowledged to cross-reactivity at immunological level between self-antigens & EBV; though, in 2003; the hypothesis of autoimmunity was proposed as the basis for human chronic autoimmune diseases with the EBV-infected B-cell reactivate [10].

This hypothesis suggests, susceptible individuals at genetic level of EBV-infected autoreactive B cells seed the target organ where they yield autoantibodies and provide co-stimulatory survival signals to T-cells autoreactive which would otherwise die by activation-induced apoptosis in the target organ place. It is assumed that autoimmunity evolves in the subsequent steps: (1) deficiency of (CD8+ T-cell); (2) The EBV primary infection ; (3) (CD8+ T-cell) control of EBV is decreased; (4) EBV load increased and increased antibodies of anti-EBV; (5) target organ EBV infection; (6) EBV-infected B-cells autoreactive clonal expansion of in the target organ; (7) into the target organ infiltration of T-cells autoreactive; and (8) ectopic lymphoid follicles development of in the target organ [11].

The Rubella virus seems to be able to infect Beta-cells directly, as revealed by in vivo and in vitro revisions. Under culture circumstances the human islets are also predisposed to direct rubella infection. The islets of human fetal faced to rubella virus contained rubella viral antigens in both (beta & non-beta cells) and had dropped production levels of insulin, even though without any obvious cytopathology.

It is probable that the virus may inserted, exposed, or altered antigens in the cell membrane of the infected host as it buds through the cell membrane.

Antigens of Rubella virus on beta cells or rubella virus-changed antigens on the beta cells surface may be considered as foreign by the host's immune system, leading autoimmunity to specifics beta cell, and the linking of Rubella virus with other auto-immune disorders still with unknown reasons [12].

Otherwise, numerous revisions provide proof for an interaction between the acquired immune responses to HCMV and appearances of some autoimmune sicknesses, for example, in RA or SLE. Precisely, immunopathogenic process by which HCMV could donate to the autoimmune disease

course, for instance, molecular mimicry by (UL83 / pp65) in SLE patients, in addition to joint inflammation by induction and expansion of (CD4+ / CD28− T-
cells) in HCMV infected RA patients. Upcoming studies should deliver comprehensive details insight into the immuno-pathological potential of viruses-reactive immune cells to develop probable new strategies of targeted therapeutic interference or resolved this issues [2].

5. Conclusion

Our data support an association of the presence of autoimmune diseases with viral infection by the high level of virus's Seropositivity that had been detected. In addition to, this study propose that viruses have the ability to induce the autoimmunity disorders by molecular- mimicry and their other effects during beginning of disease.

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