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The relationship between Rheumatoid Arthritis and prevalence of Cytomegalovirus and *Helicobacter pylori*

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Abstract: *The pathogenesis of rheumatoid arthritis (RA), related to a majority of inflammatory and autoimmune disease, is largely due to an inappropriate or inadequate immune response to external factors, among these factors, infectious agents (Cytomegalovirus and Helicobacter pylori)which are considered as candidates of triggering such disease. Method:40 RA patients and 20 healthy control individuals were recruited and tested for Rapid H.pylori and CMV IgG, all RA patients were also tested for quantitative RA IgG, IgM and IgA. Results: the results showed an elevated concentrations of RA IgG , IgM and IgA compared to the normal value of less than 20 U/ml , results also showed no significance differences in the percentage of positive result for H.pylori between RA patient 27.5% and control group 25% , whereas the concentration of CMV IgG in RA patient was significantly higher (110 U/ml) compared to control group (25 U/ml) at ($p \leq 0.01$) .In conclusion; CMV show more prevalence than H. pylori in the study population of some Iraqi patients .*
Key words: *Rheumatoid Arthritis, Cytomegalovirus and Helicobacter pylori.*

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I. Introduction

Autoimmune diseases (AID) is a complex deregulation of immunity, resulting in loss of self-tolerance and subsequent effect on endogenous tissue or cells. Onset of autoimmunity depends both on genetic and environmental factors (e.g., viruses or bacteria) and is typically driven by antibodies or T-cells reacting against self-epitopes.

Rheumatoid arthritis (RA) pathogenesis, is largely due to an inadequate immune response in genetically predisposed individuals to external factors, with bacteria and viruses (latent carrier or clinical signs of infection) being the most important triggers among these external factors (1).The microbial concept of RA triggering agents has been discussed since the 1870s (2), but in spite of this long history, a direct role of microbes in the disease is debatable, while the suspected pathogen list is still growing. It is impossible to make a direct link between a specific pathogen and the disease, as a result, there is necessity for larger studies with the use of more advanced techniques (3). In rheumatoid arthritis (RA), disturbances in the T cell pool and associations with polymorphisms in T cell genes strongly implicated in disease pathogenesis. (4,5)

Cytomegalovirus (CMV) is a viral group known as herpesviruses,a doublestandard DNA virus . The species that infects humans is commonly named human CMV (HMCV) or human herpesvirus-5(HHV-5),CMV has known to have the ability to remain latent within the body fora long time.Cytomegalovirus (CMV) infection causes clinically severe infections in immunocompromised patients and spendan influence on the peripheral , circulating T cell pool in healthy individuals without clinical disease. The involvement of the peripheral T cell pool in the CMV induced immune response ,which has been called memory inflation and which can be observed in both CD4_ and CD8_ T cells (6,7). As a result, anti-CMV seropositivity is associated with accelerated immunosenescence of CD4_ and CD8_ T cells in otherwise healthy individuals (8) and shows several parallels with the premature aging of the immune system that is a hallmark of RA. In patients with RA, an association of anti – CMV seropositivity with the expansion of CD4_CD28null T cells has been established in a number of studies (9,10). *Helicobacter pylori*, previously *Campylobacter pylori*, a gram negative, microaerophilic bacterium established usually in the stomach. It is also related to be the cause of duodenal ulcers and stomach cancer. However, over 80% of individuals infected with the bacterium are asymptomatic, it also plays an important role in the environment of natural stomach (11), others suggest that more than 50% of the world's population have *H. pylori* in their upper gastrointestinal tract (12). Infection is more prevalent in developing countries and related with different clinical disesea (13). Since the identification of *Helicobacter pylori* in the stomach (14), this microorganism has been subjected as a causative factor in the formation of gastro duodenal mucosal lesions (15), and the removal of *H. pylori* has been found to be effective for the treatment of such lesions (16).

Rheumatoid arthritis (RA) is a disease which requires the long-term use of NSAIDs. Because of this, patients with RA frequently develop gastro duodenal mucosal lesions. However, the correlation between *H.*

pylori and gastro duodenal lesions in RA patients remains controversial (17,18). In order to verify the impact of *H. pylori* infection and CMV in patients with RA and to determine whether *H. pylori*or CMV has any significance with respect to the clinical features of RA, we investigated the prevalence of *H. pylori* and CMV in a number of patients with RA.

II. Materials and methods

1- Subjects :

Blood samples **(60)** were collected from Al Yarmouk general hospital and Baghdad medical city hospital under supervision of a specialist physician , **(40)** samples with positive result of **RA** and **(20)** healthy control individuals were recruited in this study.

Blood was left to clot into plane tubes, after that serum was separated by centrifugation and carefully the serum was transfer to new labeled tubesand were frozen at **(-20°C)** until used .

2. Anti-helicobacter :

Serum samples were left to get warm and back to its liquid state.When the specimen was ready, the pouch was opened at the notch and the device was removed. (The device was placed on clean and flat surface), the device was labeled with specimen's ID number .The plastic dropper was filled with specimen.Add **30-45 µl** of specimen into the sample well.Add **30-45 µl** of sample diluent to the sample well.Results were read in 15 minute.

3. The ELISA (enzyme linked immunsorbent assay) for CMV:

All specimens and reagent were left to reach room temperature **(25 °C)**, sample andcalibrators**(10 µl , 35 µl ,100µl,200µl**). The microtitraton strips were marked,the serum sampleswere diluted 1:101 by distributing 10 **µl** of serum into 1ml of sample diluent. **100 µl** of each diluted serum sample and ready to use calibrators were transferred to the convenient wells. The plate was incubated for 30 minutes at **37°C** .Wells were aspirated and washed three times for 30 second with wash solution. Then **100 µl** of ready to use Enzyme –labeled 2nd Antibody-conjugate was added into each well.The plate was incubated again for 30 minute at **37°C,**then aspirated and washed again as above and **100 µl** of **TMB**chromogen solution was added to each well (including the blank) .The plate was incubated for 15 minutes at room temperature (without the exposure into sun light), then **100 µl** of stopping solution was added to each well. Results were read within 30 minute using microplate reader set to **450 nm**.

Statistical Analysis:

The statistical analysis system (SAS) program was used to detect difference factors in study parameters. T-test was used to compare significance between means and Chi-square test was used to compare significance between percentage of groups that were studied in the present study, $p<0.05$ considered significant.

III. Results and Discussion

In this study 60 blood specimens were collected , 40 Rheumatoid Arthritis (RA) blood specimens and 20 from healthy. control. individuals.

Their ages ranged between (21-63) years, from the 40 RA patients there were 30 female and 10 males, whereas, in the 20 healthy control there were 11 female and 9 males who matched in age with RA patients group .

1. Age and gender distribution :

The data resulting from this study showed a high prevalence of RA patients in age group ranging between (20-29) years with a percentage of (37.5%) as shown in Table (1).

Table (1) : Distribution of RA patients according to age .

Age group (years)	No.	%
20 - 29	15	37.5
30 - 39	8	20
40 - 49	4	10
50 - 59	6	15
>60	7	17.5
Total	40	100

The resultshowed a higher percentage (75%) of females with RA, compared with the percentage of males (25%) as shown in Table (2).The age at disease onset is important since younger patients refer their RA to pervious infection more often than did older patients (18).

Table (2) : Distribution of RA patients and controls accordingto gender.

Gender	RA	%	Control	%
Male	10	25	9	45
Female	30	75	11	55
Total	40	100	20	100

2. Rheumatoid IgG , IgM and IgA :

Rheumatoid arthritis patients with positive Rheumatoid factor test were also tested for specific rheumatoid IgG ,IgM and IgA concentration. Result showed high mean concentration of all IgG, IgM and IgA compared to the normal concentration which is >20 U/ml, as shown in Table (3).

Table (3) : Rheumatoid IgG , IgM and IgA in RA patients.

	Mean conc. U/ml	
	Rheumatoid arthritis	**Normal**
IgG	90.8	<20
IgM	182.4	<20
IgA	75.8	<20

This indicate an abnormal concentration in specific Rheumatoid Immunoglobulin, the body is making high levels of antibodies that is making rheumatoid patient antigens.

3. *Helicobacter pylori* relation to RA :

Rapid *H.pylori* test was performed for all study group (RA patients and control), result showed no significant increase in the percentage of positive *H.pylori* for RA patients (27.5%) compared to the percentage of healthy control group (25%) , as shown in Table (4) , Fig (1).

Table (4) : number and percentage of positive Rapid *H.pylori* test for both RA patients and control.

	Rapid *H.pylori* positive test	No.	%
RA	11	40	27.5
control	5	20	25

P>0.05, NonSignificance.

These result were in agreement with some studies,whom showed that the correlation between *H.pylori* and gastro-duodenal lesion in RA patients remain controversial (19) .Studies also referred to those *H.pylori* infections influenced neither gastro-duodenal mucosal lesion nor the clinical features ofarthropathy in patients with RA (20).

According to epidemiological and clinical investigations, the differences in the prevalence of *H.pylori* infection between RA patients and general population insignificant(21).

Some histological analysis revealed that patients with positive *H.pylori* finding had greater mucosal atrophy than those with negative *H.pylori* findings this seems to be compatible with large amount of data a for non – RA, because amyloidosis is a life – threatening complication of RA (22), Other studies suggest that *H.pylori* is implicated in the pathogenesis of RA , that it's removal may induce a significant improvement of disease activity(23).

The infection does not have an aetiological role, but it may contribute to maintain an inflammatory status in response to the continuous antigenic stimulus induced by chronic infection. The host immunological and inflammatory response against the bacteria is detected and determined by the direct or indirect production of various cytokines (24).

4. Cytomegalovirus (CMV) relation with RA :

The cytomegalovirus antibodies was detected quantitatively in all study individuals. Results reveled a significant increase in the concentration of IgG of CMV in RA patients group (110 U/ml) compared to control group (25U/ml) at ($p<0.05$), as shown in Fig (2) .[Standard Curve showed in Fig (3)].

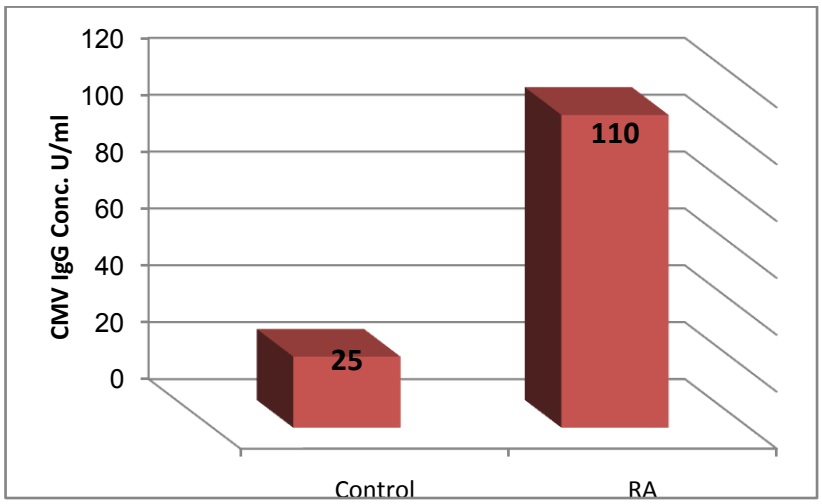
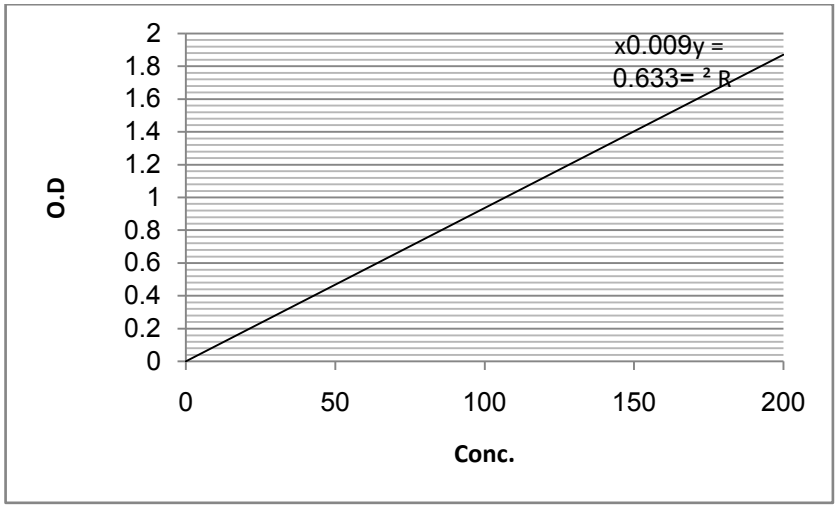


Fig (2): Concentration of CMV IgG in RA patients and control group.



Fig(3) : Standard curve of CMV IgG.

These result were in agreement with other studies (25).Cytomegalovirus (CMV) infection causes clinically sever infection in immunocompramised patients. Anti-CMV positive samples are associated with increasedimmunosenescence of CD4+ and CD8+ T-cell in otherwise healthy individual (26) , and shows several parallels with the premature aging of the immune system that is a hallmark of RA . The differences in trigger factor might show changes in the pathogenic mechanism.

RA is recognized as being a multi gene disorder with a very large number of genetic polymorphism contributing to the disease pathogenesis and cytokine production control (27). The differences in trigger factor may also be due to geographic life style , drug chemical exposure and ethnic differences (28) .

Molecular mimicry occurs when foreign antigens bear sufficient structural similarity to self-antigens. As a result, an immune response to pathogens could result in a cross-reactivity with self-antigens(29). In the view of inflammation a variety of bacterial proteinsgo through several different types of post – translation modification, and a large number of these proteins have motifs similar to those found in human polypeptide.

IV. Conclusion

The perfect immune system response to bacterial or viral invasion is based on principles for ideal balance between all members of the immune system, However in some individualsdisruption of such balance can lead to the development of RA according to many factorsincludingsusceptibility to bacterial and viral infectionin some RA patient's greater than other individuals, the imbalance of the immune system greater than

other individuals and inflammatory reaction become uncontrolled which lead to RA development,as well as genetic and epigenetic problems.

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