# Study the Role of HLA-DQB1 Alleles and Interlukine-17 in Women with Rheumatoid Arthritis in Al Najaf Governorate

# دراسة دور الأليلات (HLA-DQB1) والأنترلوكين 17 لدى النساء المصابات بالتهاب المفاصل الرثوي في محافظة النجف

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**الخلاصة**

**الهدف:** تهدف الدراسة الى تحديد العلاقة الوراثية بين أليل التطابق النسيجي (**HLA-DQB1**) مع التهاب المفاصل الرثوي, وقياس مستوى الأنترلوكين 17 وعلاقته بالأضداد المناعية الخاصة بالتهاب المفاصل الرثوي .

**المنهجية:** هذه الدراسة شملت 60 امرأة مصابة بالتهاب المفاصل الرثوي ممن زرن مدينة الصدر الطبية في محافظة النجف للفترة من شباط 2015 الى تموز 2015 وقد تم تأكيد التشخيص بواسطة استخدام فحوصات الأضداد المناعية الخاصة بالمرض وتشمل ( **Anti-CCP ,RF**) وشملت الدراسة 60 امرأة صحيحة كمجموعة سيطرة , وقد تم تحديد أليلات التطابق النسيجي **HLA-DQB1 alleles** للمجموعتين بواسطة **MR. Spot (SSO System)** وتم ايضا قياس مستوى الانترلوكين 17 في الامصال للمجموعتين بواسطة استخدام تقنية الاليزا . هذه الدراسة صممت لدراسة الحالات والسيطرة وقد تم تحليل النتائج باستخدام الوسائل الاحصائية (**SPSS version 20**) , **t-test groups** , **Chi square test** ,**Pearson correlation(r)**.

**النتائج**: نتائج هذه الدراسة اظهرت بأن تكرارية أليل **HLA DQB1*6** في مجموعة المرضى هي اكثر تكرار حيث بلغت **73.3%** واظهرت (**OR=3.85**) اي ان هذا الأليل يمثل عامل خطورة للنساء الحاملين له فيكن اكثر عرضة للإصابة بالتهاب المفاصل الرثوي بحوالي **3.85** من المرات بينما في مجموعة السيطرة اظهر أليل (**HLA-DQB1*3**) اكثر تكرارا (**66.6%**) والذي قد يكون كعامل حماية من المرض. اظهرت الدراسة ايضا ارتفاع مستوى الأنترلوكين 17 في امصال النساء المصابات بالتهاب المفاصل الرثوي حيث سجلت فارقا معنويا كبيرا (**P value < 0.001**) عند المقارنة بمستواه في امصال مجموعة السيطرة . بالإضافة الى ذلك وجود ارتباط كبير بين وجود الأضداد المناعية لل (**CCP** ) و (**RF** )والزيادة في مستوى الانترلوكين 17 في مجموعة المرضى.

**الاستنتاج**: هذه الدراسة أستنتجت أهمية تحديد الأليل**HLA-DQB1*6** كعامل خطورة للمرض وامكانية التنبؤ بحدوث المرض للنساء الحاملات لهذا الأليل. أهمية استخدام فحوصات قياس مستوى الأنترلوكين 17 لدى مرضى التهاب المفاصل الرثوي وذلك للدور المهم في تقدم المرض وعلاقته بالأضداد المناعية الناتجة من التهاب المفاصل الرثوي .

**التوصيات** : اجراء دراسة لباقي أليلات التطابق النسيجي لبيان دورها مع التهاب المفاصل الرثوي وكذلك دراسة بحجم عينات اكبر مع قياس مستوى الأنترلوكين 17 في فترات زمنية مختلفة من المرض مع دراسة أنترلوكينات اخرى مع المرض .

**Abstract**:

**Objectives**: The study aim to detect the genetic relationship between HLA-DQB1 alleles with Rheumatoid Arthritis and measurement level of Interleukin 17 and it is relationship with immune antibody that related with Rheumatoid arthritis.

**Methodology:** this study included 60 women have Rheumatoid arthritis whom visited Al-Sadder Medical City in Al Najaf Governorate from February 2015 to July 2015 , and confirmed the diagnosis by use specific antibody test related with disease include (Anti-CCP) Anti Cyclic Citrullinated Peptide, (RF) Rheumatoid Factor. This study included 60 women as healthy control group, and HLA-DQB1allele has been identified for both group by MR. Spot (SSO System). Interleukin 17 was detected in serum for both group by using ELISA Technique, this study designated as case control study and the results was analyzed by using statistical methods (SPSS version 20,t-test groups, Chi square test, persons correlation -r)

**Results:** the result of this study showed that frequency of HLA-DQB1*6 in patients group more frequent about 73.3% and showed (OR=3.85) that mean this allele represent as risk factor for women whose carried it that mean more susceptible for infected with Rheumatoid arthritis about 3.85 times, while in control group showed HLA-DQB1*3 more frequent (66.6%) that may as a protective factor from disease. This study showed increasing in level of Interleukin 17 in serum of women have Rheumatoid arthritis and that registered high significant differences (P<0.001) when compare with level in serum of control group. Moreover there is significant correlation between presence of (anti-CCP) Anti Cyclic Citrullinated Peptide and RF(Rheumatoid Factor) antibodies with increasing in level of Interleukin 17 in patient group.

**Conclusions:** this study concluded that important of detection HLA-DQB1*6 as a risk factor for disease and possible to prognostic for inducing disease for women have that allele. Using of Interleukin 17 test with Rheumatoid arthritis patients that may due to important role in progressive of disease and associated it with producing antibodies from Rheumatoid arthritis.

**Recommendations:** perform further study for other HLA alleles to clarify role that allele with Rheumatoid Arthritis, also study with larger sample size for detecting level of IL17 through different times of disease and study other interleukin with Rheumatoid arthritis disease.

**Keywords: Rheumatoid Arthritis,(HLA) Human Leukocyte Antigen , Interleukin 17, anti-CCP, Rheumatoid factor.**

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# INTRODUCTION

Rheumatoid arthritis is a chronic autoimmune inflammatory disease which affecting multi articular of synovial tissue, Moreover Rheumatoid arthritis lead to produce inflammation of the synovial membrane around the joints and swelling (hyperplasia) of synovial cell, increase in the synovial fluid and induce development of fibrous tissue in the synovium, The process of disease lead to induce destruction in articular cartilage **(1).**

RA initiate primarily by innate immune mechanisms including macrophage and mesenchymal cells that causes inducing immune responses in the synovial membrane **(2).** The interaction between T cell and B cell that follow grade of inflammation , these interaction lead to produce autoantibodies , immune complex and accretion in activation of immune cells**(3).**

The genetic factors associate with pathogenesis of RA have been detect in about 60% and the more genetic factors that related with RA is certain locus of human leukocyte antigen (HLA). Some studies referred to association between certain shared epitope(SE) alleles with RA cases have positive anti CCP **(4)( 5).** HLA-DQ is a part of HLA class II ,it is located as surface of cell protein receptor on APCs. DQ consist of two chains include α and β, these chains lead to form two loci include DQA1 and DQB1 by encoding that chains through HLA . These two loci founded in arrangement beside each other on a part of chromosome 6 p21.3. HLA-DQ loci mostly have related with HLA-DR, but its relation with HLA-DP, HLA-A, HLA-B,HLA-C is less than relation between DQ and DR **(6)**.

IL17 is one of the important proinflammatory cytokine produced by a different immune and non-immune cells (CD4+ Th17 T cells), that play important role in the pathogenesis of certain autoimmune disease such as Rheumatoid arthritis, that IL17 family classified to different members including IL17 A to IL17 F**(7) (8).**High level of IL17 usually associated with degree of severity for RA patients **(9).**The CD4+ T cells have role in producing of IL17 in synovial fluid of RA patients and there are several events lead to regulated chronicity of disease by role of IL17 some of them include epigenetic events caused promote in RA patients that causing excess in production of IL17 , that aid in support of continuous inflammation **(10).**So the current study was designed to track the role of IL17 and other associated factors in RA patients in Najaf Governorate.

# OBJECTIVES

The study aim to detect the genetic relationship between HLA-DQB1 alleles with Rheumatoid Arthritis and measurement level of Interleukin 17 and it is relationship with immune antibody that related with Rheumatoid arthritis.

## PATIENTS AND METHODS:

**1. Patients**: The study was conducted from February to July 2015 on women whom visited AL-Sadder Medical City in Al Najaf Province that included 60 women whom diagnosed as RA patients by specific diagnostic test for disease which includes Anti-CCP and RF.

**2. Control:** sixty women as healthy control group who had no history or clinical evidence of Rheumatoid arthritis disease.

**3. Collection of samples**: Six ml. veni puncture blood sample were collected under a septic condition from both patients and healthy controls and divided into two part the first included 2 ml was added into EDTA tube to use for DNA extraction , second included 4ml. used for serum collection by centrifugation at 1500 rpm. Then the serum was kept in deep freeze (-20 C) that can be used for detect anti-CCP ,RF and IL17 by ELISA test (Enzyme Linked Immune Sorbent Assay).

**4**.**Serological test:** included

Anti-Cyclic Citrullinated Peptide (Anti-CCP) and Rheumatoid Factor (RF)performed by using Aeskulisa kit/ Germany by ELISA instrument.

Other test: Interleukin 17 was performed by usingMyBiosource kit/USA(ELISA kit) .

**5. Molecular test (HLA Typing) Principle of Assay**

this assay comprised from four steps:

-DNA extraction

- amplification of extracted DNA by PCR
- hybridization and finding
- interpretation of data

For every whole blood sample was DNA extraced by using commercial kits , in the next step the extracted DNA was amplified through specific locus in the PCR reaction , that used MgCl2 solution and mastermix which available with HISTO SPOT kits. the amplification of DNA was specified by used set of biotinylated primers which specific for present HLA locus. Subsequently, the product from PCR amplification in plate was carried to the MR.SPOT processor.

The hybridization buffer was added into each test well, this well implicate specific probes called sequence- specific oligonucleotide ( SSO). There are different type of probes such as single oligonucleotide probes or combination probes , some probes such as Mosaic probes which aid in evolve the determination of cis located polymorphism.

The principle of reaction due to binding between biotin encoded amplicon with specific sequence implied in SSO probes and can determine by colourimetric reaction. While non-specific binding of the amplicon was blocked by add block buffer prior to transporting the amplicon.

Non bounded amplicon will remove by adding stringent wash, and then phosphate buffer (conjugate) was added into each well for binding with biotin that encoded of amplicone. Then substrate was added to each well that follow more wash step, addition substrate into well and effect of alkaline phosphatase lead to produce blue- purple colour.

MR. SPOT have ability to take photograph for each test well that contain colored dots in the bottom and these photo was sent to HISTO MATCH software for analysis , detection spot intensity and compare it with controls .

After that analysis and detection, the results were determined for positive and negative reactions. Finally, the HLA type of each sample was detected depend on specific hybridization pattern and that detect performed by specific program for pattern matching in HISTO MATCH software.

## RESULTS:

The demographical distribution reveals that the average age of patients was 40 to 60 years old with mean (48.31±5.524) ,while the average age of healthy controls was 40 to 59 years old with mean (48.45±5.26) Table(1)..

Regarding the body weight of RA patients, this study showed the mean of body weight (71.98±8.52) Kg was reported in cases group, while the mean of body weight (75.15±9.61) Kg was reported in healthy group and P value (0.059) which is indicated no significant differences between cases and healthy groups.

Regarding duration of disease, this study was showed the mean of disease duration (2.9±1.1) years was reported in RA patients.

The occurrence of Anti CCP antibodies showed highly significant difference between cases and control groups , and these antibodies occur in cases group with mean of (296.55±288.05) while in healthy control group have mean of (6.401±2.66) Table(1).

This table showed high significant differences in level of RF when comparison between cases and control groups ,where in cases group have mean of (131.83±119.57) while in control group have mean (17.08±44.43) .

The table (1) showed highly significant differences (P<0.001) when compare level of IL17 between RA cases and control groups.

**Table (1) Demographic and some markers in cases and control groups**

| Variable | Control(n=60) Mean±SD | Cases(n=60) Mean±SD | P value |
|---|---|---|---|
| **Age/years** | 48.45±5.26 | 48.31±5.524 | 0.893 |
| **Body weight/kg** | 75.15±9.61 | 71.98±8.52 | 0.059 |
| **AntiCCP** | 6.401±2.66 | 296.55±288.05 | <0.001* |
| **RF** | 17.08±44.43 | 131.83±119.57 | <0.001* |
| **IL17** | 101.33±61.69 | 632.7±308.61 | <0.001* |
| **Duration of disease** | - | 2.9±1.1 | |

***Highly significant**

This table showed no significant differences between two group in age and body weight distribution, and showed highly significant differences in level of anti-CCP,RF and IL17.

**Level of anti-CCP antibody:**

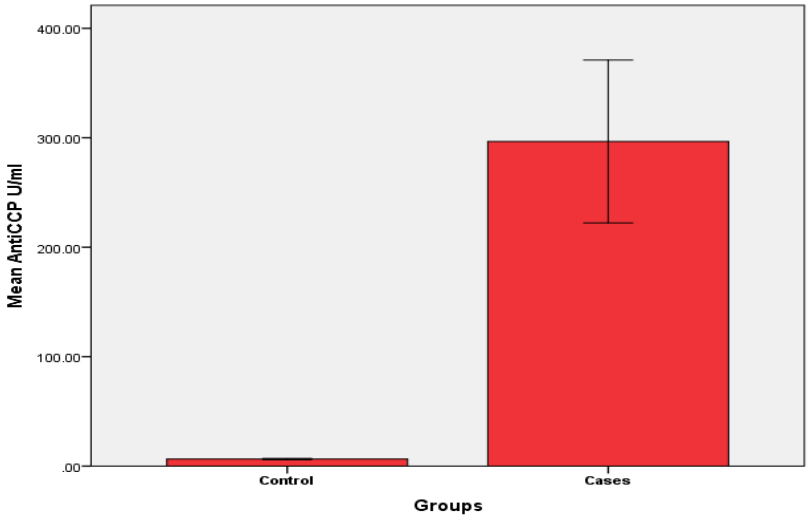


**Figure (1) Anti CCP level in RA cases and controls**.

The levels of anti-CCP antibodies were high significantly in RA cases (296.55±288.05 U/ml) in comparison with control group(6.401±2.66 U/ml). Figure (1)

**Level of RF in studied group:**

The detection RF in sera of suspected patients consider as one of diagnostic marker for RA disease. it has been demonstrated by utilize quantitative method for detection RF has been used for each sample. The level of RF in sera of RA patient was significantly higher than control. Figure (2)

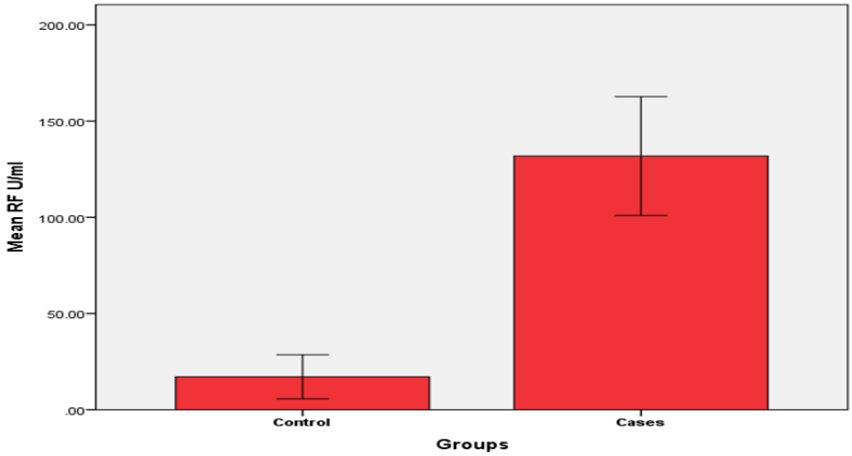



**Figure (2) level of rheumatoid factor in RA cases**

**HLA –DQB1 Genotyping result:**

HLA DQB1 was performed in RA cases and control group by using SSO system .

**Table (2) HLA DQB1 Allele in different groups.**

| HLA DQB1 Allele | RA (n=60) | % | Control(n=60) | % | OR(95%CI) |
|---|---|---|---|---|---|
| 2 | 18 | 30% | 21 | 35% | 0.79(0.34-1.8) |
| 3 | 32 | 53.3% | 40 | 66.6% | 0.57(0.25-1.27) |
| 4 | 6 | 10% | 5 | 8.3% | 1.2(0.3-4.9) |
| 5 | 20 | 33.3% | 29 | 48.3% | 0.53(0.23-1.19) |
| 6 | 44 | 73.3% | 25 | 41.6% | 3.85(1.67-8.97) |

The table (2) shows HLA –DQB1*6 was more common allele among RA cases (73.3%) and this allele had 3.85 times tendency for RA that lead to consider HLA-DQB1*6 as a risk factor allele for RA disease. While HLA-DQB1*3 was more frequent allele in control group (66.6%) that indicated may be a protective factor for RA disease.

**Interleukin-17 level of RA cases and control groups:**

The present study showed that there is significant difference between RA group and healthy control group in level of IL17 which showed ( 632.7 ± 308.61pg/ml) in RA group when compares with control group was showed (101.33±61.69pg/ml) Figure (3).

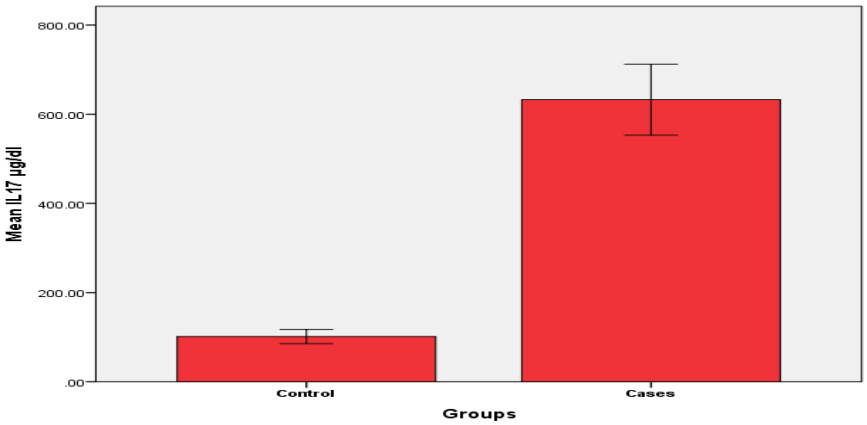



**Figure (3) level of IL-17 among studied groups.**

**Correlation between RF and RA disease:**

There was a strong significant correlation (p<0.001) between the presence of RF with Rheumatoid disease when compare the significant differences between cases and control groups. Table (3)

**Table (3) Association between RF and rheumatoid disease**

| | | Groups | | P value | OR(95%CI) |
|---|---|---|---|---|---|
| | | Cases | Control | | |
| RF | Positive | 47<br>78.3% | 7<br>11.7% | <0.001 | 27.37<br>(9.19-85.65) |
| | Negative | 13<br>21.7% | 53<br>88.3% | | |
| Total | | 60<br>100.0% | 60<br>100.0% | | |

This table showed high significant association between RF and RA disease.

**Correlation between IL17 and diagnostic markers of RA**:

The present study showed moderate significant correlation with Anti-CCP antibody (P<0.001) and showed positive significant correlation with RF (P<0.001) with the level of IL17 .Fig (4)(5)

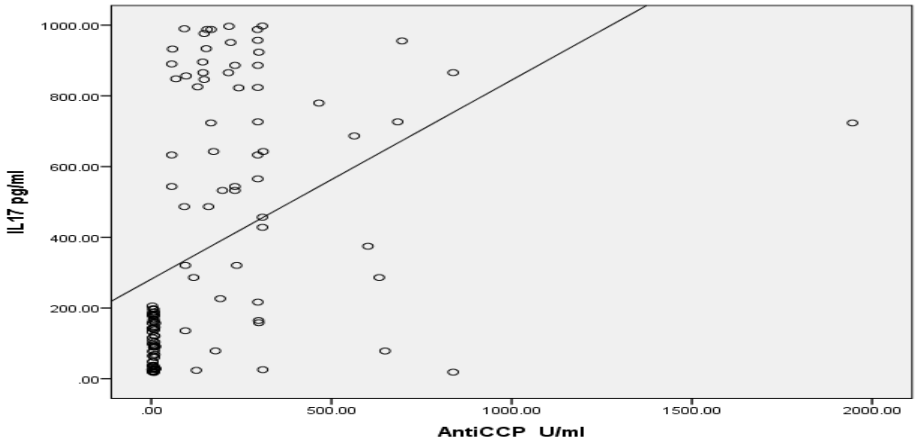
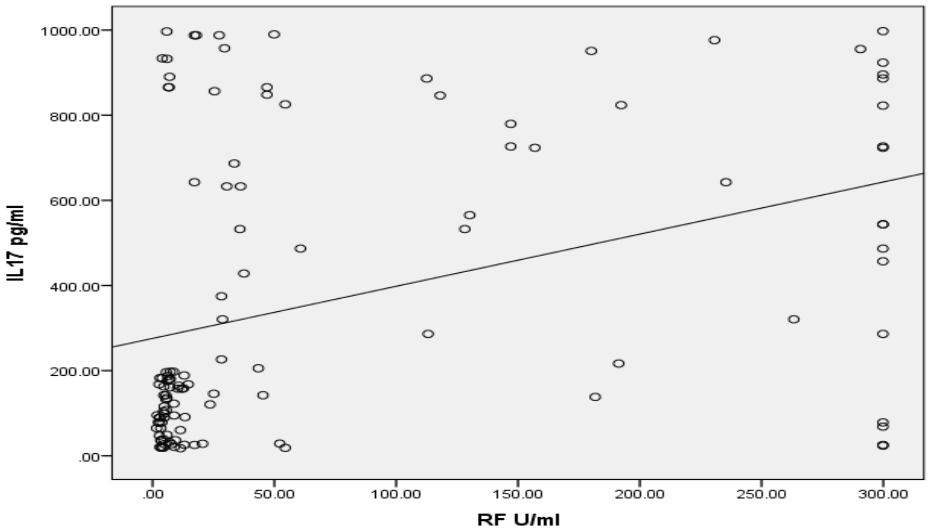



**Figure (4) Correlation between Anti CCP and IL-17**

r=0.405 P<0.001




r=0.377 P<0.001

**figure (5) Correlation between rheumatoid factor and IL-17**

## DISCUSSION:

Regarding mean of Age came nearly agree with other local previous study by Abdul-Abbas,(2007),but higher than other local previous study was reported by Hasaneen,(2007) showed mean of age (38.43 ±8.28)[11] [12] . this study was revealed the mean of age less than other European studies which some reported mean of Age among Spanish RA patients (49±2.5) years[13]**.** Therefor most of RA patients in this study were observed in age group between 40 to 60 years that was agreement with previous study performed by Silman and Pearson, 2002.these finding showed the incidence of RA disease occur between the fourth and sixth decade and that may due to fact the life span of Iraqi lower than European and that lead to incidence of disease are lower too[14]**.**

The body weight in this study showed no significant differences between two group that came disagree with other previous study reported by Wamedh et al.,2013Who indicated that body weight was significantly differences between RA cases and healthy group which P value(P≤0.05) [15]**.**

The duration of disease in this study that less than duration was mentioned from other previous study which showed (9.6 ± 8.3) years by Khalid et al., 2013. that may due to nature of life and associated chronic disease [16]**.**

The finding of level of anti-CCP near from other study done by Vasishta, (2002) who demonstrate about 80% for RA patients and about 5% for healthy controls that is positive for anti-CCP[17] . these finding that due to fact of high specificity of anti-CCP antibodies for diagnosis of RA disease and aid in differentiation from other form of Rheumatoid disease .

The level of RF in sera of RA patient was significantly higher than control. Figure (2) and become increasing with more severe cases of RA disease . RF antibodies showed 78.3% positivity for RA patients group that indicated the positivity of RF was higher than that of other study was performed by Wilson (2006) who mentioned the level of RF was elevated about seventy percent

in RA patients[18], these differences may be occur according to life nature of population and timing of sample collection .

Regarding all studies that carried out on correlation of all types of HLA alleles with frequency of RA, the present study was showed high frequency of HLA-DQB1*06 among RA patients which occur with 44 cases from 60 RA patients (73.3%) while in control group occur in 25 cases from 60 healthy group, as well as this study can indicated there is significant increase of HLA-DQB1 *06 in RA patients when compare with control group. While, the frequency of HLA –DQB1*03 was showed high in control group (66.6%) more than other alleles that may consider as a protective allele from RA disease. These result was more consistent with result reported by Ali et al.( 2006) who referred that , HLA-DQB1*6 as a genetic factor for predisposing of RA disease[19]. that result may due to fact HLA DQ is one of the important genetic components that can association with RA disease and their alleles varies according to racial of population and geographical location [20].

These observation was consistent with other previous studies which showed the important role of IL17 in some autoimmune disease especially with RA disease [21]. Other study by Zhang et al. showed that elevation in level of IL17 and IL22 in RA patients when comparison with osteoarthritis patients and healthy controls [22].Moreover other study was reported that increase of IL 17 levels in RA patients and role of that cytokine in pathogenic of disease [23] [24]. These finding may due to fact that IL17 have a main role in stimulate other proinflammatory agents and aid in accumulation of dendritic cells, monocytes , neutrophil and TNFα that lead to inducing of inflammation then progress of the disease to reach destruction of joint [25].

These result come consistent with other study reported by Anca et al,(2012 ) who showed a strong correlation between level of IL17 in serum and specific markers for RA disease which include anti-CCP and RF[26] ,moreover these finding come nearly from other study reported by Kokkonen et al,(2010) who mentioned increase in level of IL17 in RA cases and come significant with diagnostic marker for disease[23]. In these finding of present study may be due elevation of IL17 levels in RA patients according to influence of systemic response to inflammation which usually associated with autoimmune disease [27].

## CONCLUSION

**The present study concluded that:**

**1.** Anti-CCP and RF have an important role in diagnosis and prognosis of RA disease.
**2.** The human with HLA DQB1*6 are more susceptible to infection with RA.
**3.** A significant correlation between IL17 and RA disease that may help in the confirmation of the RA activity.

## RECOMMENDATION:

**1.** Further survey studies with large sample size are needed to make large data base for RA.
**2.** Study other type of HLA allele with RA disease.
**3.** Study the role of other Interleukin with Rheumatoid Arthritis disease such as IL6 and IL10.

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