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RESEARCH ARTICLE

ASSOCIATION BETWEEN HLA-DRB1 ALLELES AND SUSCEPTIBILITY TO RHEUMATOID ARTHRITIS AND TREATMENT RESPONSE.

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Abstract

Background and Objectives: Rheumatoid arthritis is an autoimmune disease with poorly understood pathophysiology. Genetic component of disease etiology, especially human leukocyte antigen (HLA) association, is well known. Ethnic differences account for a number of variations in disease association with the HLA locus and there seem to be differences in various studies regarding the role of HLA in genetic predisposition to disease. This work was aimed to study Human Leukocyte antigen HLA-DRB1 alleles association with susceptibility to rheumatoid arthritis and relation between certain alleles and response to treatment. **Patients and method**s: The study included 48 rheumatoid arthritis (RA) patients diagnosed on the basis of criteria established by the American College of Rheumatology(ACR), and 48 sex matched healthy volunteers as a control group. HLA-DRB1 Typing using Luminex X-Map technology was done for all patients and controls. **Results**: frequency distribution of HLA-DRB1 alleles was compared between patients and controls, a significant difference was demonstrated as regards frequency of HLA-DRB1*04 and HLA-DRB1*16 alleles being higher among patients compared to controls. HLA-DRB1*04, *13 alleles were significantly associated with high disease activity. HLA-DRB1*15 and DRB1*11 alleles were significantly associated with good response to treatment in the form of disease modifying antirheumatic drugs (DMARDs) , while HLA-DRB1*04, DRB1*01 and DRB1*16 alleles were significantly more common among non responders to treatment or deteriorated patients. **Conclusion**: Our study suggests that HLA-DRB1*04 and DRB1*16 alleles are susceptibility alleles to RA. HLA-DRB1*04 and *13 may be of prognostic value as they were significantly associated with high disease activity indicating a high risk for developing a more active type of the disease. HLA-DRB1* 11, *15, *04, *01 and *16 may be predictors for response to treatment.

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Introduction:-

Rheumatoid arthritis (RA) is a chronic, progressive, inflammatory autoimmune disease associated with articular, extra-articular and systemic effects. It has been reported that RA affects ~0.5–1% of the adult population of developed regions, and in Egypt prevalence is about 0.3% **(Thierry et al., 2014)**.

It results from a complex interaction between genes and environment, leading to a breakdown of immune tolerance and synovial inflammation in a characteristic symmetric pattern **(Firestein et al., 2017).**

Susceptibility to RA involve genetic factors and is modulated by environmental and non inherited factors **(Stahi et al., 2010) .** Genetic factors accounts for about 40% to 60% of the total risk **(Ucar et al., 2012).**

The most important genetic factor implicated in predisposition to RA is human leukocyte antigen (HLA) major histocompatability (MHC) genes, HLA-DR4 is the major genetic factor implicated but its relative importance varies across ethnic groups **(Okada et al., 2012).**

HLA gene products play a key role in antivirus and antitumor defense, They function in regulation of immune response to foreign antigens and discrimination of self from non- self antigens. Alloantigens are taken up by antigen presenting cells which process them and re-express the antigens on the cell surface along with HLA to be recognized by T-cell receptor **(Powell et al., 2012).**

The extremely high levels of polymorphism and heterozygosity within the MHC genomic region provide the immune system with a selective advantage against the diversity and variability of pathogens . However, the high level of polymorphisms and mutations in the MHC has the added risk of generating autoimmune diseases and other genetic disorders. **(Deitiker, Atassi 2015).**

There is an association between HLA-DRB1 alleles and RA susceptibility (shared epitope hypothesis) (SE), HLA-DRB1*04:04, 04:01, 01:01, 10:01 and 14:02 alleles play a key role in predisposition to disease. They encode specific amino acid sequence (Q/RK/RRAA) in position 70-74 of third hypervariable region of β chain, Some other alleles HLA-DRB1 *01:03, 04:02, *12, *13:01, *13:02, *13:04 carrying another sequence of amino acids D/ERAA in third hypervariable region of β chain are considered protective. **(Bax et al., 2011).**

Approximately 30-40% of RA patients do not have a good response to treatment despite optimal dosing regimen **(Maillefert et al., 2010).** Also, some patients develop important side effects such as cytopenias, gastrointestinal adverse effects or abnormal liver function tests which may limit use of treatment, So it is a great deal of interest to discover biomarkers of treatment response to DMARDs such as HLA genotype **(Kooloos et al., 2011).**

Patients And Methods:-

Patients: This case control study was carried out in Rheumatology & Clinical pathology department of Zagazig University Hospital during the period from June 2016 to December 2017. It was carried out on 48 rheumatoid

Arthritis (RA) patients diagnosed on the basis of criteria established by the American College of Rheumatology(ACR) **(Aletaha et al., 2013)** , and 48 sex matched healthy volunteers as a control group. Patients suffering from other rheumatological diseases were excluded from the study. The study protocol was approved by the institutional ethics committee and written informed consent was obtained from all participants

The patients were subjected to: i) full clinical history and thorough clinical examination. ii) Estimation of disease activity according to DAS28 Score. iii) routine laboratory investigations, including Complete blood count, Liver and Kidney function tests, rheumatoid factor (RF) and C-reactive protein CRP assay and ESR measurement. iiii) Specific laboratory investigation: All patients and controls were subjected to molecular HLA-DRB1 Typing by Luminex X-Map technology.

HLA typing. Using a Luminex LABScan 100 and LAB Type SSO Class II DRB1 Typing Kit. LAB Type SSO is based on reverse SSO technique, Biotinylated amplicon is chemically denatured and the separated strands are allowed to rehybridize to complmentary DNA probes conjugated to fluorescently coated beads. This is followed by removal of unbound material by wash solution, After wash, Streptavidin conjugated Phycoerythrin (SAPE) is added which binds to any biotinylated hybridized product, this is followed by wash step again to remove unbound

substance, then a flow analyzer LAB scan 100 is used to identify the fluorescent intensity of (PE) on each microsphere. **(Deshpande et al., 2010).**

EDTA-anticoagulated whole-blood samples were collected from patients and controls and processed as follows:

1. ***DNA* extraction** using the spin column technique (QIAamp DNA Blood Mini kits;Qiagen, Hilden, Germany) was performed according to the manufacturer's guidelines;
2. **Measuring concentration of extracted DNA** was done by Qubet fluorometre by using specifically formulated dye that binds selectively to dsDNA to minimize the effects of contaminants in the sample then detection of target-specific fluorescence, and the target concentration needed to begin amplification reaction was adjusted to 20ng/µl.
3. **polymerase chain reaction (PCR) amplification of the HLA-DRB1 gene (exon 2) target** (LAB Type SSO class II HLA-DRB1 Typing kit; Hannover, GERMANY) was performed and Presence of amplified product was confirmed by gel electrophoresis through visualization of band at 270 bp under UV transluminator.
4. **Denaturation/ Neutralization** the amplicons were chemically denatured by denaturation and neutralization buffer to form single stranded DNA.
5. **Hybridization:** combination of appropriate volume of bead mixture (LAB Type SSO Bead Mix; Hannover, GERMANY) with hybridization buffer according to manufactures guide-lines.
6. **Washing**: of hybridization bead mixture was done by wash buffer for a total of 3 washes.
7. **Labelling:** The conjugate Streptavidin conjugated phycoerythrin (SAPE) was added, streptavidin binds to the biotin of the PCR product and Phycoerythrin is a fluorescent dye used for labeling by binding to any biotinylaed hybridized product.
8. **Washing:** was done again for labeled hybridized product.
9. **Injection and reading on Luminex:** a suspension of bead mixture with wash buffer was injected on Luminex.

Interpretation of results:-

A flow analyzer, the Luminex LAB Scan 100 identifies the fluorescent intensity of PE (Phycoerythrin) on each microsphere The assignment of the HLA type is based on the reaction pattern compared to pattern associated with the published HLA gene sequence. The microsphere mixture consists of a set of fluorescently labeled microspheres that bear unique sequence-specific oligonucleotide probes for HLA alleles. Each microsphere mixture includes negative and positive control microspheres for substraction of non-specific background.

The mean fluorescence intensity (MFI) generated by Luminex software contain the FI for each bead (or probe bound to bead) per sample. The percent positive value is calculated as:

Percent positive value= 100* {(MFI (probe n)-MFI (probe negative control)/MFI (probe positive control)-MFI (Probe negative control)}.

1. The MFI for negative control should be 0-100.
2. The MFI for positive control should be 1200-7000 MFI.
3. The HLA allele or allele groups of the sample is determined by matching the pattern of positive and negative
4. beads with the information in the LAB Type SSO worksheet.

Disease activity assessment:-

Disease activity for RA patients was evaluated by the Disease Activity Score28 (DAS28) (**Prevoo et al., 1995).** (DAS28) consists of a 28 tender joint count (range 0-28), a 28 swollen joint count (range 2-28), ESR and global Health (GH) on a Vas scale (range 0-100). DAS28 is a continuous index ranging from 2 to 10. DAS28 is calculated by using the following formula based on tender joint count (TJC), swollen joint count (SJC), Patients global health (GH) , and either CRP (mg/L) or ESR (mm/h):

$DAS28 = 0.56*\sqrt{(TJC28)} + 0.28*\sqrt{(NSJ28)} + 0.7*(ESR) + 0.014*(GH)$.

Grading of activity according to DAS28 Score: (Dougados et al., 2005).

1. Low disease activity is defined as DAS28 < 3.2.
2. Moderate as 3.2< DAS28 < 5.1.
3. Sever activity as DAS28> 5.1.
4. DAS28 < 2.6 corresponds to remission.

Patients were classified into 5 groups according to treatment protocol given:

1. Methotrexate with antimalarial drugs.
2. Methotrexate with prednisone.
3. Salazopyrine with prednisone.
4. Methotrexate only.
5. Avera.

Patient's improvement and response to treatment was evaluated by measuring the change in two consequtive DAS scores, one was calculated at the time of the study and the other from previous records. Patients were classified into 3 groups according to degree of improvement; moderate improvement, stable and deteriorated

Statistical Analysis:-

All data were collected, tabulated and statistically analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as the mean ± SD & median (range), and qualitative data were expressed as absolute frequencies (number) & relative frequencies (percentage). t test was used to compare between two groups of normally distributed variables. The Kruskall Wallus test was used to compare between more than two groups when they were not normally distributed. Percent of categorical variables were compared using Chi-square test and Fisher Exact test when appropriate. All tests were two sided. p-value < 0.05 was considered statistically significant, ≥ 0.05 was considered statistically insignificant.

Results:-

The general Characteristics of the study patients and control group are shown in (table 1). Clinical and laboratory data of our patients are shown in (table 2 and table 3).

Table 1:-Demographic characteristics of both groups

Variables	patients(no=48)	control(no=48)	Test of sign	p- value	Sig
Age (years)					
$\overline{X}$ ±SD Median (range)	47.5±8.03 48 (30-61)	47.7±8.08 48 (30-62)	t=0.14	0.8	**NS**
Sex					
females /males ratio	46/2 (23)	45/3 (15)	-	*0.9	**NS**
Duration of disease					
(years) $\overline{X}$ ±SD Median (range)	6.5±2.6 6 (2-10)				

Table 2:-Clinical manifestation of rheumatoid arthritis patients (n=48)

Variables		
Extra-articular manifestation n=27 (57%)	**Number**	(%)
1. Dry eye &mouth(Sjogren's syndrome)	15	(31.2)
2. Skin ulcer (vasculitis)**.**	2	(4.2)
3. Cough, expectoration, chest tightness	5	(10.4)
4. Tingling, numbness in extremities (peripheral neuropathy).	4	(8.3)
5. Subcutaneous nodules	1	(2.9)
Number of tender joint		
$\overline{X}$ ±SD Median (range)	9±6 9 (0-19)	
Number of Swollen joint		
$\overline{X}$ ±SD Median (range)	**6±**5 6 (0-16)	

Table 3:-Laboratory Data of the studied patients.

Complete blood picture	All studied patients (no. 48)		
	Mean± SD	**No. (%)**	**Median (Range)**
Hemoglobin (g/dl)			

Normal (12-16) g/dl Mild anaemia (10-12) Moderate anaemia (7-10)	11±1.15	12 28 8	(25%) (58%) (17%)	10.95 (9 – 14)
Platelets ($x10^3/mm^3$)				
Normal (150-400) Mild Thrombocytopenia(100-150) Thrombocytosis > 400	256±105	40 5 3	(83.3%) (10.4%) (6.3%)	237 (125 –531)
WBC ($10x^3/mm^3$)	7.2±2.5	----		6.3 (4–13)
Liver functions	**All studied patients (no. 48)**			
	Mean ± SD	**Median**		**(Range)**
ALT (U/L)	30.9 ±26	22		(10– 135)
AST (U/L)	31 ±32	22		(12– 176)
Total protein (gm/l)	7.3 ±0.6	7.4		(6.2– 8.6)
Albumin (gm/l)	3.6 ±0.4	3.7		(2.4– 4.3)
Total – Bilirubin (mg/dl)	0.42 ±0.23	0.4 (0.3-1.4)		
Direct – bilirubin (mg/dl)	0.12 ±.1	0.1 (0.1-0.6)		
Kidney Functions				
Urea (mg/dl)	23 ±15	18		(11– 67)
Creatinin (mg/dl)	0.5 ±0.09	0.6		(0.4– 0.8)
Activity markers of the studied group	**All studied patients (N=48)**			
	Mean± SD	**No. (%)**		**Median (Range)**
ESR 1st	55±24	-		50 (15 – 120)
CRP				
≤5mg/l >5mg/l	3.1±1.5 25±31	18 (37.5%) 30 (62.5%)		2(2-5) 12(6-122)
RF				
≤15 u/ml >15u/ml	9.3±1.2 180.6± 173	7 (14.6%) 41 (85.4%)		10(8-650) 90.9(20.5-650)
Total RF	155±172			84.5 (8-650)

The frequencies of HLA-DRB1 alleles in RA patients and normal controls are summarized in (**table 4)**. HLA-DRB1*04 and HLA-DRB1*16 alleles were statistically more frequent in rheumatoid arthritis patients compared to controls, p values (0.01, 0.02, respectively), Odds ratios (2.5, 5.0, respectively), whereas HLA-DRB1*07,*11 and *13 alleles were more frequent in controls than patients (ORs were 0.4, 0.7 and 0.6, respectively) but the difference in each case didn't reach a statistically significant level (p>0.05 for each). The relation between HLA-DRB1 alleles and activity markers of the disease as CRP and RF is shown in (**table 5 and 6)**. HLA-DRB1*03 and HLA-DRB1*16 alleles were found to be statistically more frequent among seronegative patients ((RF ≤ 15 U/ml) than seropositive group (RF > 15 U/ml) (p values were 0.03, 0.02) respectively, Odds ratios were (0.22 and 0.16) respectively, while no significant association was obtained between any of HLA-DRBI alleles and CRP levels in rheumatoid patients. (P>0.05).

Table 4:- HLA-DRB1 allele frequencies in patients and controls.

HLA-DRBI	Cases (48) No (%)	Controls (48) No (%)	χ 2	P	Odds(95%CI)
***01**	9 (9.4)	5 (5.2)	1.4	2.3	1.97(0.5-7.7)
***03**	14 (14.6)	10 (10.4)	0.96	0.3	1.5(0.6-4)
***04**	25 (26)	12 (12.5)	6.3	0.01(S)	2.58(1.14-5.9)
***07**	3 (3.1)	7 (7.4)	F	0.3	0.4(0.8-1.92)
***08**	0	2 (2)	F	0.2	-
***09**	0	2 (2)	F	0.2	-
***10**	0	2 (2)	F	0.12	-

***11**	11 (11.5)	15 (15.6)	0.5	0.4	0.7(0.3-1.8)
***12**	0	5 (5.2)	F	0.059	-
***13**	12 (12.5)	19 (20)	1.5	0.2	0.6(0.26-1.4)
***14**	0	4 (4.2)	F	0.12	-
***15**	13 (13.5)	11 (11.5)	0.3	0.58	1.27(0.5-3.2)
***16**	9 (9.4)	2 (2)	5	0.02(S)	5(1-49)
Total	96 (100)	96 (100)			

f= Fisher exact test. HLA, human leukocyte antigen; OR, odds ratio; CI, confidence interval.

Table 5:-Statistical comparison of HLA-DRB1 allele's distribution between seropositive and seronegative rheumatoid arthritis patients

HLA-DRBI alleles	**Rheumatoid factor**		***p**	**Odds ratio 95%CI**
	Patients n= 41	Patients n= 7		
	>15 U/ml	≤15 U/ml		
	No %	**No %**		
***01**	9 (11)	0	0.34	-
***03**	9 (11)	5 (35.7)	0.03(S)	0.22 (0.5-0.97)
***04**	23 (28)	2 (14.3)	0.3	2.4 (0.44-16)
***07**	3 (3.7)	0	0.9	-
***11**	8 (9.8)	3 (21.4)	0.19	0.4 (0.08-2.2)
***13**	12 (14.6)	0	0.2	-
***15**	13 (15.8)	0	0.2	-
***16**	5 (6.1)	4 (28.6)	0.02(S)	0.16 (0.03-0.99)
Total	82 (100)	14 (100)		

***Fisher Exact test**

Table 6:-Statistical comparison of HLA-DRB1 alleles distribution between patients with high CRP level > 5 mg/l and patients with low CRP level < 5 mg/l.

HLA-DRBI	**CRP**		***p**
	Patients n= 30	Patients n= 18	
	>5mg/dl	≤5mg/dl	
	No %	**No %**	
*01	5 (8.3)	4 (11.1)	**0.7
*03	8 (13.3)	6 (16.7)	**0.88
*04	16 (26.7)	9 (25)	*0.8
*07	3 (5)	0	*0.3
*11	8 (13.3)	3 (8.3)	*0.5
*13	7 (11.7)	5 (13.9)	*0.7
*15	10 (16.7)	3 (8.3)	*0.3
*16	3 (5)	6 (16.7)	*0.07
Total	60 (100)	36 (100)	

***fisher exact test **chi square test**

The relation between HLA-DRB1 alleles and disease activity (according to DAS28 Score) is shown in **table (7)**. A statistically significant association was found between HLA-DRB1*04 and DRB*13 alleles and disease activity score , as they were associated with the highest activities among HLA-DRB1 alleles. (p= 0.03).

Table 7:-Comparison between HLA-DRBI alleles as regards means of disease activity score (DAS) of patients.

HLA-DRBI	**Disease activity score of rheumatic arthritis patients**	^P
	$\bar{x}$ ±SD	
***01**	5±1.5	0.03 (S)
***03**	4.4±1.8	
***04**	5.6±0.95	
***07**	6.5±0	

***11**	4±1.9	
***13**	5.6±1.5	
***15**	4.2±1.7	
***16**	5.4±1.1	
Total	5 ±1.6	

^Kruskall-Wallus test. **HLA, human leukocyte antigen**

Types of treatment given to RA patients are summarized in **table (8).** The relation between HLA-DRB1 alleles frequencies and response to treatment is shown in **table (9).** A statisticaly significant relation was identified between each of the following alleles; HLA-DRBI *01,*04,*11,*15 and *16 and response to treatment (p was <0.05 for each). The best response to treatment was in patients carrying HLA-DRBI*15, followed by DRB1*11 alleles, p values (0.0005, 0.005) respectively, Whereas The worst response was detected in patients carrying HLA-DRBI *04 , DRB1*01, and in those with DRB1*16 alleles (p values 0.001, 0.009 and 0.02) respectively,

Table 8:-Types of treatment (Disease modifying antirheumatic drugs DMARDs) given to rheumatoid arthritis patients.

Treatment	**All studied patients (N=48)**	
	No	**(%)**
Methotrexate and antimalarial	25	52
Methotrexate and Predinsone	10	21
Salazopyrine and Predinsone	7	15
Methotrexate	3	6
Avera	3	6

Table 9:-Relation between frequencies of HLA-DRB1 alleles and response to treatment by DMARDS in rheumatoid arthritis patients.

HLA-DRBI	**Response**			χ^2	**P**
	Moderate improvement No (%)	**Stable No (%)**	**Deteriorated No (%)**		
*01	2 (22)	4 (45)	3 (33)	9.2	0.009 (S)
***03**	5 (36)	4 (28)	5 (36)	4.2	0.09
***04**	3 (12)	2 (8)	20 (80)	7.5	0.001(S)
***07**	0	0	3 (100)	2.4	0.29
***11**	8 (73)	0	3 (27)	10.8	0.005(S)
***13**	2 (17)	2 (17)	8 (66)	1.3	0.49
***15**	10 (77)	0	3 (23)	14.8	0.0005(S)
***16**	0	0	9 (100)	7.2	0.02(S)
Total	30 (31)	12 (13)	54 (56)		

S= significant. HLA, human leukocyte antigen.

Discussion:-

Rheumatoid arthritis (RA) is a chronic autoimmune disease with articular and systemic manifestations, It affects about 0.5-1% of adult population, mostly middle aged females (**Thierry et al., 2014)**.

The pathogenesis of rheumatoid arthritis results from complex interaction between genes and environment leading to breakdown of immune tolerance**. (Okada et al., 2012)**.

The significant association of particular alleles with RA is not consistent in all human populations in different geographical areas or among different ethnic groups, hence the analysis of different alleles of HLA in RA patients of many populations and geographical areas with regard to either having protective role or being a susceptibility factor is necessary (**Sandoughi et al., 2011).**

This study was carried out on 48 rheumatoid arthritis (RA) patients admitted to Rheumatology department of Zagazig University Hospitals during the period from June 2016 to December 2017, in addition to 48 sex matched healthy

volunteers as a control group.

The aim of the study was to assess Human Leukocyte antigen HLA-DRB1 alleles association with susceptibility to rheumatoid arthritis and relation between certain alleles and response to treatment.

Upon comparing frequency distribution of HLA-DRB1 alleles between patients and controls, a significant difference was demonstrated as regards frequency of HLA-DRB1*04 and HLA-DRB1*16 being higher among patients compared to controls (p= 0.01 and 0.02, respectively), (Odds ratios= 2.5 and 5, respectively) which suggests that HLA-DRB1*04 and*16 could be a specific susceptibility alleles for rheumatoid arthritis.

Rest of alleles had no significant differences between patients' and control groups. However, it is important to note that some alleles in the present study were found to be more frequently prevalent in controls than patients, e.g HLA-DRB1*07,*11 and *13 (ORs were 0.4, 0.7 and 0.6, respectively) despite that the difference in each case didn't reach a significant level (p>0.05 for each).

Soliman et al. 2016 studied HLA-DRB1 allele frequencies in a group of 40 Egyptian patients with RA. They have reported higher frequencies of HLA-DRB1 *01,* 04 and *10 in RA patients compared to controls, where HLA-DRB1*04 was having the highest frequency among their patients' group than in controls (p value 0.01 , odds ratio 4.5).

In addition, a study by **Saghafi et al. (2014**) on 177 patients diagnosed with RA from north east of Iran reported that HLA-DRB1*01,*03 and *04 were the most frequent alleles among patients' group, Meanwhile, frequencies of HLA-DRB1*11,*13 alleles were significantly higher in controls compared to patients (p= 0.001, OR=0.27 and P=0.001, OR=0.26) respectively which were suggested to be protective factors.

Mourad and Monem (2013) found that HLA-DRB1*01,*04 and *10 allele frequencies were higher in a group of Syrian RA patients than controls (p=0.02 and OR=2.2, p<0.00001 and OR=3.16, p=0.02 and OR=2.4, respectively). Based on their results, alleles suggested to be protective included HLA-DRB1*11 and *13 alleles, which have shown higher frequencies among controls than patients (p=0.004 and OR= 0.49, p=0.002 and OR=0.32) respectively.

Moreover, HLA-DRB1*10 has been suggested to be a susceptibility allele for RA by **Sandoughi et al., (2011)** in patients from Southeast of Iran and by **Muazzam et al., (2013)** in Pakistani population. The former group has also found a trend of positive association of RA with HLA-DRB1*04 and *16 but was not significant. It is worth noting that HLA-DRB1*04 and *16 were significantly associated with disease in the present study.

On the other hand, DRB*03 has been suggested as a protective allele by **Sandoughi et al., (2011**) as well as DRB1*11 by **Muazzam et al., (2013).**

The results of the present study , as mentioned before, has shown a trend of negative association of DRB1*11 and *13 to RA which might point to their significance as protection alleles, however they didn't reach a statistically significant value. The significant finding of a negative association of DRB1*11 and *13 to RA by other studies, as by **Muazzam et al., (2013) (**DRB1*11 only), **Mourad and Monem (2013) and Saghafi et al. (2014)** supports our finding which might require a larger number of patients to reach a stastistically significant level.

In a study carried out in Romania, HLA-DRB1*04 has shown to be a susceptibility allele in addition to DRB1*01and*14 **(Loredana et al., 2012)**. In Morocco, HLA-DRB1*04 has also been reported as a susceptibility allele while DRB1*07 as a protective allele (**Atouf et al., 2008).**

Apart from HLA-DRB1*04, the obvious discrepancy between the results of different studies, including our results, as regards frequency distribution of HLA-DRB1 alleles among RA patients can be attributed to the differences in population race, geographical distribution and ethnic origin.

The association between HLA-DRB1 alleles and activity markers of the disease as CRP and RF was assessed. By comparing HLA-DRB1 allele distribution between seropositive and seronegative patients, we found that HLA-DRB1*03 and HLA-DRB1*16 alleles were found to be statistically more frequent among seronegative patients ((RF $\leq$ 15 U/ml) than seropositive group (RF > 15 U/ml) (p values were 0.03, 0.02) respectively, Odds ratios were (0.22 and 0.16) respectively. This might point to a possible protective effect of HLA-DRB1* 03 and HLA-DRB1*16

alleles against high serum RF level.

In the present work the frequency of HLA-DRB1*04 allele was higher in RA patients with high RF levels (28%) than in patients with low levels (14.3%), but the difference didn't reach a statistically significant level (OR=2.4, P= 0.3), a finding which could be better defined among Egyptians on studying larger number of patients. In other populations, several previous studies reported the association between HLA-DRB1*04 and high RF level such as that carried out by **AL-Timimi et al., (2014)** who confirmed the association between HLA-DRB*01 and DRB1*04 alleles and RF positive Kurd patients, where 28.9% of their patients carrying DRB1*01 alleles were RF positive compared to 5.6% who were RF negative and 22.3% of patients having DRB1*04 alleles were RF positive compared to 5.6% who were negative (ORs= 5.9 and 4.8, respectively). Similar findings were also reported by **Atouf et al. in 2008** in Moroccan patients (HLA-DRB1*04 allele) and **Ucar et al. in 2012** in Turkish Patients. They reported that HLA-DRB1*04 allele has a role in sever form of rheumatoid arthritis.

On the other hand, **Kinikli and associates (2003**) reported no association between alleles and seropositivity of RA in Turkish population.

As regards serum CRP, our results showed no statistically significant association between any HLA-DRB1 alleles and CRP level in rheumatoid arthritis patients.

Considering the relation between HLA-DRB1 alleles and disease activity (as assessed by DAS28 Score), our results showed a statistically significant association between HLA-DRB1*04 and DRB1*13 alleles and disease activity, implying that they were associated with the highest activities among HLA-DRB1 alleles according to DAS28 Score (p= 0.03).

Our results partially match with those described by **Soliman et al., (2016)** in a study on 40 Egyptian RA patients and 20 controls. They reported a significant association of HLA-DRB1* 04 (P= 0.005). They have reported also the association of HLA-DRB1*01 alleles with disease activity (P=0.002), but not DRB1*13, as was obtained in our study.

Another Egyptian study, in 2009 by **Farouk** and his companions, has investigated the effect of HLA-DRB1 alleles on outcome of rheumatoid arthritis in 29 Egyptian RA patients and 15 healthy controls. The study has reported that HLA-DRB1*01 and DRB1*04 were significantly more expressed among patients with active disease than among those with inactive disease (measured by SADI score). But, in contrast to our study, they have reported that HLA-DRB1*13 (in addition to HLA-DRB1*03, *11, *12, and *14) were significantly more expressed among inactive cases.

Meanwhile, **Fathi and associates (2008)** in a study on 60 Egyptian rheumatoid arthritis patients have reported the significant association of HLA- DRB1 *04 with high activity of the disease, but not DRB1*01 or DRB1*13 alleles.

To further enlighten the possible relation between HLA- DRB1 alleles and RA in the current study, patients were categorized into 4 groups according to level of disease activity, which were determined according to DAS28 Score and included the following groups: remission (DAS < 2.6), low disease activity (2.6< DAS < 3.2) , moderate disease activity (3.2 < DAS < 5.1) and high disease activity (DAS > 5.1) groups.

In our study, a considerable percentage of patients carrying HLA-DRB1*03, *11 or *15 (36%, 27% and 16%, respectively) were found to be in remission state. While those carrying HLA-DRB1*07,*04*,*13 or *16 alleles were found to be in severely active state, by percentages of (100%, 88%, 67% and 56%, respectively).

Al-Timimi and associates (2014) reported that 20% of their patients (Kurds) with HLA-DRB1*04 were of moderate activity and 20% were of high activity, while 3.3% of patients with HLA-DRB1*13 were of moderate activity and 8% were of high activity.

Summing the above data, it can be noticed that HLA DRB1 *04 allele remains the single allele that has been consistently reported by almost all studies, including the present one, as regards its association to disease susceptibility as well as disease activity, despite the different populations studied by different groups.

In the present work the relation between HLA-DRB1 alleles and response to treatment was assessed, patients were classified into 5 groups according to type of treatment regimen given (Disease modifying antirheumatic drugs DMARDs). 25 patients (52%) were on Methotrexate and Antimalarial Drugs, 10 patients (21%) were on methotrexate and prednisone, 7 patients (15%) were on Salazopyrine and Prednisone, 3 patients (6%) were given Methotrexate only and 3 patients (6%) were on Avera. Response to treatment was evaluated by measuring the change in two consequtive DAS scores, the first from patients records and the second at the time of the study, Patients with decrease in DAS Score from initial value > 1.2 were of good improvement, decrease in DAS < 1.2 but > 0.6 were of moderate improvement, decrease in DAS <0.6 were of no improvement.

Our results showed a statisticaly significant relation between each of the following alleles; HLA-DRBI *01,*04,*11,*15 and *16 and response to treatment (p was <0.05 for each). the best response to treatment among rheumatoid arthritis patients was in those carrying HLA-DRBI*15{10 patients (77%) were of moderate improvement}, followed by DRB1*11 alleles {8 patients (73%) were of moderate improvement} p values (0.0005, 0.005) respectively.

The worst response was detected in those carrying HLA-DRBI *04 {20 patients (80%) were deteriorated}, in those carrying DRB1*01, {3 patients (33%) were of no improvement , while 4 patients (45%) were deteriorated} and in those with DRB1*16 alleles {9 patients (100%) were deteriorated} (p values (0.001, 0.009 and 0.02) respectively, the improvement was nill in HLA-DRB1*07, but this didn't reach a significant level.

In a study performed on patients from Pakistan by **Ali et al., (2006)**, 91 RA patients had been receiving methotrexate for at least 6 months and, clinical response to methotrexate was assessed after 12-24 weeks of treatment, those showing 50% or more reduction in ESR, number of swollen joints and morning stiffness as compared to those criteria at admission were classified as responders while those showing little improvement in these parameters were called non-responders.

They found that HLA-DRB1*03 allele was significantly more common among non-responders (37.2% compared to responders (14.3%) (p = 0.004). They added that HLA-DRB1*15 was present more frequently in the non-responders (46.5%) than in the responders (40.8%) , however the difference was not significant. No other alleles showed significant association with response to treatment in their study, as they have reported.

Conclusion:-

HLA-DRB1*04 and *16 alleles appear to be susceptibility factors for development of rheumatoid arthritis in our population, patients having high frequencies of HLA-DRB1*04 and *13 were predisposed to high disease activity state. In addition HLA-DRB1* 15, *11, *04, *01 and *16 alleles may be predictors for treatment outcome in rheumatoid arthritis patients.

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