Hematoid arthritis (RA) is a chronic inflammatory disease affecting mainly the synovial joints and leading to reduced quality of life. The pathogenesis of RA is complex and is thought to be multifactorial with both genetics and environmental factors involved in disease susceptibility. One of the earliest genetic factors implicated in RA is the HLA-DRB1 gene. The association with HLA-DRB1 was found to be restricted to certain alleles namely, HLA-DRB1*04:01, *04:04, *04:05, *04:08, *01:01, *01:02, *14:02 and *10:01. These alleles encode a conserved five amino acid sequence in residues 70–74 of the HLA-DRB, commonly known as the shared epitope. It has been proposed that all RA-associated HLA-DRB1 alleles share a conserved motif of amino acid residues (QKRAA/RQRAA/RRRAA) in the hypervariable region (HVR3) of the DRB1 molecule. Indeed, the HLA-DRB1* shared epitope is classically considered...
The HLA typing of all patients met the 1987 American College of Rheumatology classification criteria for the diagnosis of rheumatoid arthritis. The HLA typing was conducted using sequence specific oligonucleotide (SSO) high definition kit LABType SSO HD (One Lambda Inc., Canoga Park, CA, USA). The HLA typing was carried out according to the manufacturer’s instructions. Briefly, the HLA typing procedure consisted of DNA extraction, amplification, hybridization, reading on a Luminex machine (LABScan 100, http://www.onelambda.com/), and interpretation using HLA Fusion software.

Autoantibodies
The RF was determined by the nephelometry method (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). An RF value greater than 10 IU/mL was considered positive. Anti-CCP was determined by enzyme-linked immunosorbent assay (ELISA) using anti-CCP ELISA (IgG) test kit (INOVA Diagnostics, San Diego, USA). A value greater than 20 U/mL was considered positive. Antinuclear antibody (ANA) was assayed using the CMIA method (DiaSorin, Saluggia, Italy) and a value greater than the 1.5 index was considered positive.

Statistical analysis
Statistical analysis was conducted using version 12.0 of the software Statistics and Data (Stata) available from StataCorp, College Station, TX, USA. Continuous data were expressed as mean and standard deviation (SD). The data was normally distributed based on the Shapiro-Wilks test. Categorical data were expressed as numbers and percentage. To investigate which of the risk factors was associated independently with the shared epitope, a logistic regression analysis was performed, with shared epitope as the dependent variable and the presence of anti-CCP antibodies, ANA, and RF as possible explanatory variables. Association between different HLA alleles was analyzed using the odds ratio (OR) with 95% confidence interval (CI). The P value was corrected by the number of alleles observed (Pc). A Pc value of less than 0.05 was considered statistically significant.

RESULTS
Of 76 consecutive patients enrolled in the study, 90% were females. The age of onset among males was significantly older than females (50.4[8.6] years vs 41.7[11.5] years, P=.0245), while the current age was not significantly different between the genders (55.1[10.4] males vs 52.1 [11.1] females, P=.3794). Most of the women were married (97%) with a mean of 7 kids per mother. Most of the patients were RF positive and anti-CCP positives (77.6% and 75%, respectively), while a smaller number were ANA positive (26.3%) (Table 1). Controls were 158 healthy Saudis of different ages (between 21 and 81 years, mean 39.5 years and median 38 years). Controls were of both genders (male=81 and female=77).

Carrying the shared epitope was associated with a significantly higher risk of developing RA (OR=2.65, 95% CI: 1.42-4.94, P=.0009) (Table 1). HLA-DRB1*04:01, *04:05, *15:01 and *16:02 were significantly associated with RA. However, after correcting for multiple testing only HLA-DRB1*04:05 remained significant (OR=3.73, 95% CI (1.61-8.96), Pc=0.016) (Table 2). On the other hand, HLA-DRB1*03:01 and *07:01 were significantly protective against RA, but after correcting for multiple testing, this significance disappeared. In the logistic regression analysis, only anti-CCP was found to be associated with the shared epitope (OR=14.51, 95% CI (1.53-137.49), P=.02 (Table 3).
DISCUSSION
In this study, we describe the demographic and HLA alleles in a group of Saudi patients with RA. Interestingly, most of our patients were females (9:1, F: M). Although this finding of a preponderance of females is in agreement with previous studies in Saudi Arabia, further investigation is needed to confirm whether this finding is a hallmark of the disease in this region, or whether it is a study bias. This is especially relevant since our studied population consisted mainly of soldiers and their families. One could possibly argue that the low prevalence of the disease in males is due to their work nature and the heavy exercise regimen they undertake. Interestingly, the age of onset in our patients was earlier than that reported in the West. However, the 10-year gap in age of onset between the two genders in the Western population is also true in our study population, with men developing the disease at least 10 years later compared to women.

An observation that might be unique to this population is that most women were married with a large number of children. This is a reflection of the typically large family size in the Saudi population. The target population in our hospital was military personnel and their families. As the mean age of onset of the females in this cohort was 41 years, most of those women were probably married.

The majority of the cases did not suffer from the severe form of the disease as shown by the low prevalence of extra-articular manifestations. RF and anti-CCP antibodies were equally high in this cohort, but not ANA.

The presence of the shared epitope strongly predicted the presence of anti-CCP antibodies. This association between the shared epitope and anti-CCP is well documented in the literature. The shared epitope positivity was significantly high

| Table 1. Characteristics of the rheumatoid arthritis population (n=76). |
|-----------------|--------|--------|
| Gender          | N      | %      |
| Male            | 8      | 10.5   |
| Female          | 68     | 89.5   |
| Total           | 76     | 100.0  |
| Married         | 74     | 97.4   |
| Single          | 2      | 2.6    |
| Extra-articular manifestations | 5   | 6.58  |
| RF positive     | 59     | 77.6   |
| Anti-CCP positive | 57  | 75.0   |
| ANA positive    | 20     | 26.3   |

| Table 2. HLA-DRB1 frequency in rheumatoid arthritis (RA) patients and healthy controls. |
|---------------------------------|--------|--------|
|                                | RA patients (n=76) | Healthy controls (n=158) | OR   | 95% CI   | P  |
| DRB1*03:01                     | 13     | 8.6    | 52   | 16.5 | 0.47 | 0.23-0.92 | .021 |
| DRB1*04:01                     | 7      | 4.6    | 2    | 0.6  | 7.58 | 1.41-75.30 | .003 |
| DRB1*04:05                     | 18     | 11.8   | 11   | 3.5  | 3.73 | 1.61-8.96 | .0004 |
| DRB1*07:01                     | 21     | 13.8   | 84   | 26.6 | 0.44 | 0.25-0.76 | .0019 |
| DRB1*15:01                     | 19     | 12.5   | 20   | 6.3  | 2.11 | 1.03-4.32 | .024 |
| DRB1*16:02                     | 4      | 2.6    | 1    | 0.3  | 8.51 | 0.83-420.50 | .0225 |

Statistically significant comparisons shown here. Pc is corrected for multiple allele testing. Data were analyzed using 2×2 table for odds ratios using cci command in Stata. Correction for P values were by multiplying the P value by the number of alleles. All of the tested alleles are in Appendix 1.
in this cohort compared to controls. This was reflected by the increased frequency of HLA-DRB1*04:01 and *04:05. The latter was only significant after correcting for the multiple allele testing. HLA-DRB1*04:05 has been shown to be associated with RA in other Asian and Arab populations. Previously, Al-Arfaj described HLA association in a cohort of 92 Saudi RA patients. The main finding was the association of HLA-DRB10; his study, however, lacked a control group. In another study from Saudi Arabia, Al-Swailem and co-workers reported an association with HLA-DRB1*04:05; they did not, however, relate this to the autoantibody profile of the patients. Thus, our study is unique in reporting a strong association between carrying the shared epitope status and developing anti-CCP antibodies in Saudi patients with RA.

In summary, we describe consecutive RA patients attending the Rheumatology Clinic at the National Guard Hospital in Riyadh. Most of the patients were married women with large families. Most of them were positive for RF and anti-CCP antibodies. The latter was strongly predicted by the presence of the shared epitope. Since

### Table 3. Logistic regression analysis of shared epitope status and the risk for developing anti-CCP antibodies, ANA, and RF.

| Predictor       | Odds Ratio | 95% CI      | P     |
|-----------------|------------|-------------|-------|
| Anti-CCP        | 14.51      | 1.53-137.49 | .02   |
| ANA             | 1.87       | 0.54-6.53   | .32   |
| RF              | 2.71       | 0.41-17.91  | .30   |

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### References

1. Strand V, Khanna D. The impact of rheumatoid arthritis and treatment on patient’s lives. Clin Exp Rheumatol. May-Jun 2010;28(3 Suppl 59):532-40.
2. Perricone C, Cecarelli F, Valesini G. An overview on the genetic of rheumatoid arthritis: a never-ending story. Autoimmun Rev. Aug 2011;10(10):599-606.
3. Sastri P. Association of the B-cell alloantigen DRw4 with rheumatoid arthritis. N Engl J Med. Apr 20 1978;298(16):869-871.
4. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum. Nov 1987;30(11):1205-1213.
5. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis & Rheumatism. 1987;30(11):1205-1213.
6. Gonzalez-Gay MA, Garcia-Fornu C, Hajee AH. Influence of human leukocyte antigen-DRB1 on the susceptibility and severity of rheumatoid arthritis. Paper presented at: Seminars in arthritis and rheumatism, 2002.
7. Holoshitz J. The rheumatoid arthritis HLA-DRB1 shared epitope. Curr Opin Rheumatol. May 2010;22(3):293-298.
8. van der Linden MP, van der Woude D, Ioan-Facsinay A, et al. Value of anti-modified citrullinated vimentin and third-generation anti-cyclical citrulline peptide compared with second-generation anti-cyclical citrullinated peptide and rheumatoid factor in predicting disease outcome in undifferentiated arthritis and rheumatoid arthritis. Arthritis Rheum. Aug 2009;60(8):2232-2241.
9. Hajee AH, Al Balwi MA, Aytul Uyar F, et al. HLA-A, -B, -C, -DRB1 and -DQB1 allele and haplotype frequencies in Saudis using next generation sequencing technique. Tissue Antigens. Oct 2013;82(4):252-258.
10. Arnett FC, Edworthy SM, Bloch DA, et al. The american rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis & Rheumatism. 1988;31(3):315-324.
11. Al-Swailem R, Al-Rays H, Sobki S, Arfin M, Tarig M. HLA-DRB1 association in Saudi rheumatoid arthritis patients. Rheumatology international. 2006;26(11):1019-1024.
12. Al-Arfaj A. HLA-DR pattern of rheumatoid arthritis in Saudi Arabia. Annals of Saudi medicine. 2001;21(1-2):92-93.
13. Cooney JK, Law RJ, Matschke V, et al. Benefits of exercise in rheumatoid arthritis. J Aging Res. 2011;2011:681640.
14. Symmons DP. Epidemiology of rheumatoid arthritis: determinants of onset, persistence and outcome. Best Pract Res Clin Rheumatol. Dec 2002;16(5):707-722.
15. Abdul Salam A. Population and Household Census, Kingdom of Saudi Arabia 2010: Facts and Figures, International Journal of Humanities and Social Science. 2013;3(16):258-263.
16. Bos WH, Ursun J, de Vries N, et al. The role of the shared epitope in arthralgia with anti-cyclical citrullinated peptide antibodies (anti-CCP), and its effect on anti-CCP levels. Ann Rheum Dis. Sep 2008;67(9):1347-1350.
17. Rojas-Villarraga A, Diaz FJ, Calvo-Parra E, et al. Familial disease, the HLA-DRB1 shared epitope and anti-CCP antibodies influence time at appearance of substantial joint damage in rheumatoid arthritis. J Autoimmun. Feb 2009;32(1):64-69.
18. Liu X, Guo J, Jia Y, et al. HLA-DRB1 shared epitope-dependent DR-DQ haplotypes are associated with both anti-CCP-positive and -negative rheumatoid arthritis in Chinese Han. PloS One. 2013;8(8):e71373.
19. Taneya V, Giphart MJ, Verduijn W, Naipal A, Malaviya AN, Mehra NK. Polymorphism of HLA-DRB, -DQA1, and -DQB1 in rheumatoid arthritis in Asian Indians: association with DQB1*0405 and DRB1*1001. Hum Immunol. Mar 1996;46(1):35-41.
20. Dhauloud T, Sfar I, Abdelmoula L, et al. Association of specific amino acid sequence (QRRAA) of HLA-DRB1*0405 with rheumatoid arthritis in a Tunisian population. Arch Inst Pasteur Tunis. 2010;87(1-2):53-59.