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Occurence of autoantibodies in healthy Omani individuals

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نسبة وجود الأجسام المناعية المضادة الذاتيةفي شريحة من المجتمع العماني

علي بن عبدالله الجابري و اليزابيث ريتشينز

الملخص: الهدف : دراسة نسبة وجود الأجسام المناعية المضادة الذاتية في شريحة من المجتمع العماني. **الطريقة :** تم مخبريا دراسة عينة من الأصحاء تتكون من ٣٩٢ فردا (١٣٨ إمرأة و ٢٠٩ رجلا) وذلك للكشف عن نسبة وجود الأجسام المناعية المضادة الذاتية. **النتائج :** أظهرت الدراسة وجود الأجسام المناعية المضادة الذاتية في حوالي ٥٠ % من العينة التى درست ولكن بتركيز ضئيل، بينما كانت نسبة وجود هذه الأجسام بتركيز عالٍ فقط في عدد محدود من الأفراد. وكانت نسبة الأجسام المناعية المضادة الذاتية للعضلات اللا إرادية هي الأعلى لدى الفئة المدروسه (٣١ر٦%) تليها الأجسام المناعية المضادة الذاتية للغدة الدرقية بنسبة ٥ر٤ % .وظهرت الأجسام المناعية المضادة الذاتية لنوى الخلايا في هذه الشريحة من العمانيين بنسبة ضئيلة ١ر٥ %، الأجسام المناعية المضادة الذاتية لاجزاء من خلايا المعدة و١ر٨ % والأجسام المناعية المضادة الذاتية لأغشية الخلايا ٣ % والأجسام المناعية المضادة الذاتية لعامل روماتويد ١ % . **الخلاصة:** أشارت هذه الدراسة وهي الاولى من نوعها في المجتمع العماني الى وجود الأجسام المناعية المضادة الذاتية لدى الأفراد الأصحاء مؤكدة نتائج مجموعة من الدراسات السابقة والتى أجريت على شرائح من مجتمعات مختلفة . وتوصي هدة الدراسة بتوخي الحرص والحذر عند استخدام هذه الأجسام المناعية المضادة الذاتية فى التشخيص الاكلينيكى لأمراض المناعة الذاتية .

ABSTRACT. ***Objectives:*** To investigate the occurence of various autoantibodies in the Omani population. ***Method:*** Sera from 392 healthy Omani individuals comprising 183 pregnant women and 209 blood donors (183 men and 26 women) were investigated. Autoantibodies were detected using immunofluorescence, haemagglutination and latex agglutination techniques. ***Result:*** Low levels of autoantibodies were detected in approximately 50% of the subjects; very few subjects showed high autoantibody titres. Anti smooth muscle autoantibodies (ASMA) were the most prevalent, and were detected in 31.6% of the individuals. Anti thyroid microsomal autoantibodies (ATMA) and anti thyroglobulin autoantibodies (ATA) were present in 5.9% and 4.9% of individuals respectively. The other autoantibodies were detected much less frequently, viz. anti nuclear autoantibodies (ANA) in 1.5%, anti parietal cells autoantibodies (APCA) in 1.8%, anti reticulin autoantibodies patterns (ARAP) in 3.0% and rheumatoid factor (RF) in 1.0% of the subjects. ***Conclusion:*** The data indicate that autoantibodies do exist in healthy Omani individuals and the results of clinical tests for these autoantibodies must be interpreted with caution.

Key Words: autoantibodies, Oman, healthy individuals, autoimmune diseases

Autoantibodies can be present in sera of patients with or without autoimmune diseases. The literature clearly defines their role in specific autoimmune conditions.[1–3] Their presence merely supports, not confirms the diagnosis of an autoimmune disease; for a clinical diagnosis, evidence for clinical disease and/or tissue damage should be present. The definition of abnormality related to autoantibody levels is usually based on abnormal quantity rather than affinity or avidity. Thus, abnormal autoantibody levels can result in clinical or sub-clinical autoimmune conditions.[2]

The demonstration and interpretation of autoantibodies in sera from patients is a diagnostic tool in autoimmune diseases. Thus, the availability of data on the frequency and strength of autoantibodies within a normal population is important for determination of diagnostic levels.[1–3] Many such population studies have been performed in healthy individuals in various communities.[5–9] However, such data have not been described for Oman. This study aimed to establish baseline data for Oman and to provide clinicians with the level of occurence of autoantibodies within this population. Thus, this is the first report on the distribution of different autoantibodies within the normal Omani population.

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METHOD

Two different groups were investigated, pregnant women and blood donors. Individuals in both the groups, totalling 392, were all healthy with no history and/or symptoms of autoimmune diseases. All were residents of the capital area (Muscat), which has the highest population density in Oman.

The pregnant group comprised 183 normal women, while the blood donor group numbered 209 individuals, 183 men and 26 women (Figure 1). All sera were kept frozen at –20°C and thawed only at the time of testing.

Tests were conducted to detect the following eight autoantibodies: anti nuclear antibodies (ANA), anti smooth muscle antibodies (ASMA), anti mitochondrial antibodies (AMA), anti parietal cells antibodies (APCA), anti thyroglobulin antibodies (ATA), anti thyroid microsomal antibodies (ATMA), anti reticu- lin antibodies patterns (ARAP) and rheumatoid factor (RF).

Indirect Quantafluor fluorescent autoantibody tests[10] were used for the detection and semiquantitation of all autoantibodies except ATA, ATMA and RF where haemagglutination and latex agglutination assays, respectively, were used.[11]

Indirect immunofluorescence (IIF)

Sera were tested at a 1:20 dilution by standard immunofluorescence techniques (Quantafluor, Kallested, USA). Positive sera were titrated to determine the relative strength of the autoantibody under test.

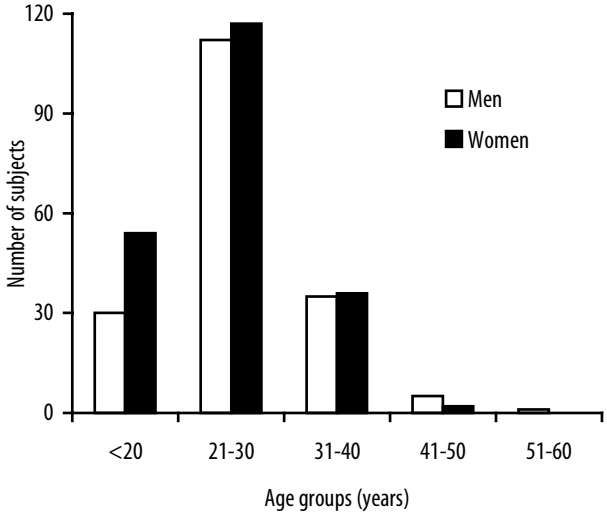


Figure 1. *Age distribution of the Omani individuals studied*

The study population, which consisted of 392 health volunteers (183 men and 209 women), was divided according to age into 5 groups.

Agglutination assays

Haemagglutination assays using turkey erythrocytes with bound microsomal antigens and bound thyroglobulin antigens were used to test for ATMA and ATA respectively (Thymune-M and Thymune T, Murex, UK). Sera were first inactivated at 56°C for 30 minutes. Positive and negative controls were included in each microtitre plate. Sera and erythrocytes were mixed then incubated at room temperature out of direct sunlight for one hour in the case

Table 1. *The number and percentage of Omani individuals positive for various autoantibodies*

Auto-antibody	Titre range		Pregnant women (F=183) *		Blood donors (M=183, F=26) ♦		Combined (n=392)	
	From	To	number positive	%	number positive	%	number positive	%
ASMA	1:20	1:160	63	34.5	61	29.2	124	31.6
ATMA	1:100	1:6400	11	6.0	12	5.7	23	5.9
ATA	1:10	1:1280	11	6.0	5	2.4	16	4.1
ARAP	1:20	1:20	3	1.6	9	4.3	12	3.1
APCA	1:20	1:640	6	3.3	1	0.5	7	1.8
AMA	1:20	1:20	0	0.0	5	2.4	5	1.3
ANA	1:20	1:20	0	0.0	6	2.9	6	1.5
RF	1:20	1:80	4	2.2	0	0.0	4	1.0
Total			98	53.6	99	47.4	197	50.3

**Age range 16–46 (Mean 26 years). ♦ Age range 17–58 (Mean 27 years); F= Number of women; M= Number of men*

ASMA: anti smooth muscle autoantibodies, ATMA: anti thyroid microsomal autoantibodies, ATA: thyroglobulin autoantibodies, AMA: antimitochondrial antibodies, ANA: antinuclear autoantibodies ARAP: antireticulin autoantibodies patterns, APCA: anti parietal cells autoantibodies, RF: rheumatoid factor

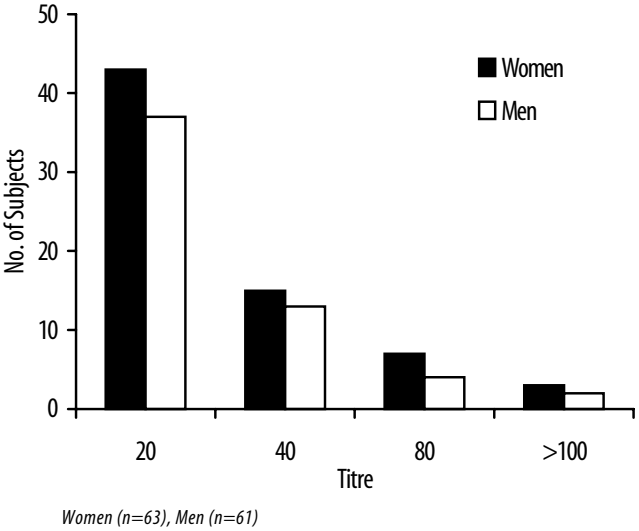


Figure 2. *The number of Omani individuals positive for ASMA at different titres.*

of ATMA and for 30 minutes in the case of ATA tests. The end point titre was taken as the highest dilution of the sample giving approximately 50% agglutination of the test cells. ATA tests were also performed by indirect immunofluorescence using monkey thyroid as a substrate (Quantafluor, Kallested, USA).

Rheumatoid factor was determined by the latex agglutination method (Humatex RF detection kit, Germany). Sera were first inactivated at 56°C and mixed with human gamma immunoglobulin coated latex particles. Macroscopic agglutination was seen in sera where RF was present. The sera were first diluted 1:20 in fresh buffer (containing glycine 0.1M sodium hydroxide 2.5mM sodium chloride 0.15M, sodium azide 0.1g/dl, pH 8.2); an equal volume of serum (50µl) was mixed with latex particles and checked for agglutination. Positive and negative controls were always included. Positive samples were titrated to determine their titres.

Table 2. *Ten pregnant Omani women positive for more than one autoantibody, with their titres*

Age	ATA	ATMA	ASMA	AMA	APCA	RF
20	1:40	1:400	–	–	–	–
21	1:1280	1:400	–	–	–	–
23	1:20	–	–	–	–	1:20
24	1:320	1:400	1:20	–	–	–
25	1:320	–	1:20	–	–	–
26	–	–	1:20	–	–	1:80
27	–	1:1600	1:20	–	–	–
27	1:20	1:400	–	–	–	–
32	1:1280	1:400	–	–	1:320	–
35	–	1:400	–	–	1:640	–

RESULTS

The results, summarized in Table 1, show that ASMA, the most frequent autoantibody present in this population, was detected in 63/183 (34.5%) and 61/209 (29.2%) of pregnant women and blood donors respectively ($p < 0.05$). However, only three pregnant women (1.6%) and two blood donors (0.9%) had titres greater than 1:100 for this autoantibody [Figure 2]. This titre is considered to be of clinical significance in chronic autoimmune hepatitis.[12–13] The remaining 60 pregnant women had low ASMA titres [Figure 2]. This was also the case for the blood donors, where 37/61 individuals had titres less than 1:20.

Thyroid antibodies occurred in 11/183 (6%) for both ATA and ATMA in the pregnant women and in 5/209 (2.4%) and in 12/209 (5.7%) of blood donors for ATA and ATMA respectively [Table 1]. Of the twelve blood donors who tested positive for ATMA, nine were men and three were women. ATMA were detected at titres ranging between 1:100 and 1:1600. All men positive for ATMA had lower titres compared to women among whom one case of 1:1600 and two cases of 1:400 were seen. For ATA, three men and two women were shown to be positive. Women showed high titre of antibodies compared to men.

The other antibodies occurred generally at lower frequencies. Thus ARAP occurred in 3/183 (1.6%) of the pregnant women and in 9/209 (4.3%) of the blood group donors; AMA were not detected in the pregnant women, but were found in 5/209 (2.4%) of the blood donors; APCA occurred in 6/183 (3.3%) and in 1/209 (0.5%) of pregnant women and blood donors; RF in 4/183 (2.2%) of pregnant women but was not detected amongst blood donors [Table 1]. ANA were absent in pregnant women but found in 6/209 (2.9%) blood donors.

Ten women (5.5%) tested positive for more than one of the autoantibodies [Table 2]. Although some of the titres were high, there were no clinical symptoms of autoimmune diseases in these persons.

Considering the two groups together, autoantibodies have been detected in more than half of the subjects studied (50.3%). Low titre autoantibodies occurred at high frequency in both groups, having been found in 98/183 (53.6%) and in 99/209 (47.4%) of the pregnant women and the blood donors respectively ($p < 0.05$); [Table 1]. The most common autoantibodies detected were ASMA (31.6%), ATMA (5.9%), ATA (4.1%), ARAP (3.1%), APCA (1.8%), ANA (1.5%), and RF (1%).

DISCUSSION

Autoimmune phenomena can occur when tolerance to self-components fails and the immune system starts to produce autoantibodies. There is considerable evidence to suggest that autoantibodies may be present in the serum of apparently healthy individuals as well as of patients with autoimmune diseases.[4–8,15–16] Many factors are known to influence the production of autoantibodies, including genetic factors, sex, age and viral infections.[14]

In the present study, two groups of healthy Omani individuals were investigated for different autoantibodies: pregnant women and blood donors. Autoantibodies have previously been described in sera from normal pregnant women[17–19] and it has been reported that there is no significant difference in the frequency of autoantibodies between pregnant and non-pregnant women.[20]

ASMA were first found in the sera of patients with chronic active hepatitis and subsequently in those with other autoimmune liver diseases, viral infection, certain cancers, heroin addiction and female infertility.[12] Now it is known that ASMA can occur at low levels in sera of healthy individuals. Our study confirms this, by showing a prevalence of 31.6% in our sample of normal Omanis [Figures 2 & 3]. Such high level may be attributed to genetic and/or environmental factors.[14] ASMA occurred at a similar frequency in both pregnant females and blood donors. Thus it appears that there is no gender difference in the frequency of ASMA in the present study.

Table 3. *Clinically significant titres of autoantibodies among Omanis*

Autoantibody	Pregnant Women	Blood Donors	Combined
ASMA	>80	>80	>80
ANA	≥ 20	≥ 20	≥ 20
ATA	> 40	> 40	> 40
ATMA	> 400	>100	>100
AMA	> 20	> 20	> 20
APCA	>20	>20	>20
ARA	>20	>20	>20
RF	>20	>20	>20

The table shows cut-off values (titres), of the different autoantibodies studied, that may be clinically significant among the Omani population.

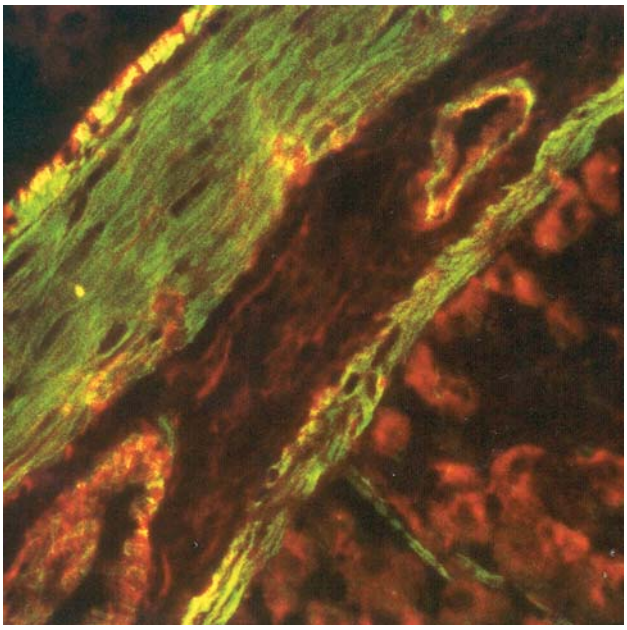


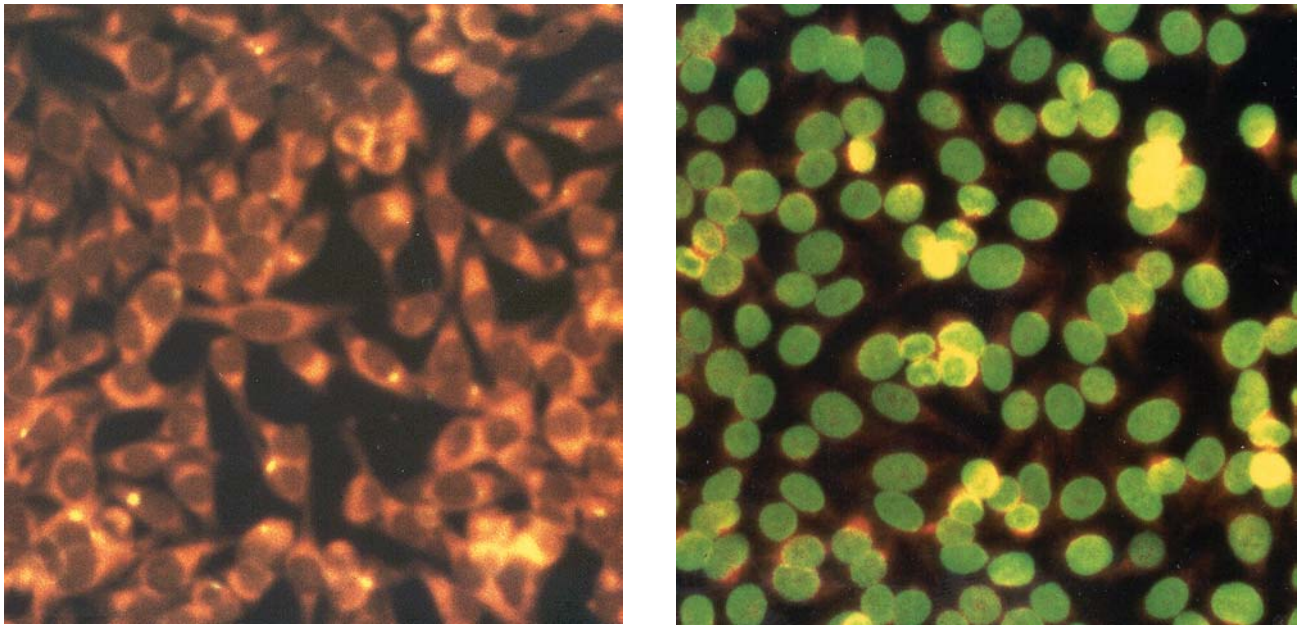
Figure 3. *Positive immunofluoresence of gastric smooth muscle*

Since the vast majority of our subjects were less than 40 years old, with only five above 40, [Figure 1], we are unable to comment on the frequency of autoantibodies in older subjects (> 40 years). This should be addressed in future studies on this population.

Baig and Shere,[8] in their study of a Saudi Arabian population, found ANA to be more prevalent in females than in males, whereas in our study only two women and four men among the blood donors and none among the pregnant women were positive for ANA [Figure 4]. The titres of ANA were all very low, at no more than 1:20 [Tables 1 & 3]; the prevalence of 1.5% of ANA in this study group is much lower than the prevalence of 4.5% found by Baig and Shere in their Saudi Arabian study.[8] However, the prevalence of other autoantibodies with high titre (ATA and ATMA) is found higher in females than in males of a matched age group. Within the blood donors group, we noted a higher frequency of autoantibodies in older subjects. These findings support those of other studies.[8, 21] Sex and age factors are important when evaluating the presence and the titre of autoantibodies within patients.[7]

ATMA and ATA are also common autoantibodies among this group (occur in approximately 5%) and they come second after ASMA. Females tend to have high titres of ATA and ATMA. ANA is not very common within this population and RF is rarely detected. Only four pregnant Omani women were positive for RF. ARAP is common within both groups.

There are many reasons for performing immunological laboratory investigations for autoantibodies. Occasionally,



a. *Human epithelial (Hep-2) cells negative for antinuclear antibodies*

b. *Antinuclear antibodies detected by Hep-2 cells*

Figure 4 . *Indirect immunofluoresence for the demonstration of antinuclear antibodies*

a diagnosis can be made from a positive test. More commonly the test may contribute significantly to diagnosis, confirm a clinical diagnosis or exclude a possible diagnosis and are valuable for monitoring treatment. Low levels of many autoantibodies are not of diagnostic importance and may occur in otherwise normal individuals.[4–6] For example, prevalence of autoantibodies increases steadily with age,[7] and low titres of autoantibodies in the elderly are frequently of no clinical significance. In general, both autoantibodies and autoimmune diseases can be found more frequently in woman, particularly Caucasians, than in men.[3] Due to such variations, data on the frequency and strength of autoantibodies in normal individuals in a community are essential as benchmarks for interpreting the autoantibody results of patients from that community. In the absence of this knowledge, such tests may not help in the management of patients. Physician seeking help from a laboratory without this knowledge must be resorting to the last refuge of the diagnostically destitute.

The results of this study show clearly the existence of autoantibodies within normal healthy individuals as has been already shown by other investigators.[4–8,22] The existence of autoreactive antibodies, both in healthy subjects and in patients with autoimmune diseases, is a consequence of intra-thymic selective processes during the development of the T cell repertoire. Exposure of self-antigens to the thymic environment during foetal life results in the elimination of specific anti-self T cells; the dominant mechanism is physical, that is, clonal deletion. Autoreactive T cells against antigens which do not passage through the thymus during developmental stages may be eliminated via functional inactivation i.e. clonal anergy.[23] Failure of either of these processes will result in the maturation of autoreactive T cells.[24] There are many mechanisms which result in the breakdown of self tolerance. They include alteration in the control of apoptosis, cross reactivity and molecular mimicry, anti-idiotype antibodies that function as autoantibodies and the polyclonal stimulation of natural autoantibody-producing cells that then progress via mutation and isotype switching.[25]

These phenomena emphasize the importance of distinguishing between autoimmune parameters and autoimmune disease. The former is benign whereas the latter is potentially fatal. Autoimmunity often reflected only by the presence of autoantibodies, a normal consequence of aging and readily inducible by drugs or infectious agents and potentially reversible, i.e., it may disappear when the offending drug or agent is eradicated. Autoimmunity may be a normal physiological state: we are all probably autoimmune, but relatively few of us develop autoimmune diseases. These result when auto-reactive T and B cells, activated by genetic environmental triggers, cause actual tissue damage.[26]

The development of autoimmune disease is dependent on at least four factors. The two major ones are genetic and viral. A third factor is endocrine; oestrogen promotes autoimmune disease, whereas androgen acts as a natural immunosuppressive agent. These physiological effects of sex hormones on the normal immune response explain the marked female predominance of autoimmune diseases on susceptible genetic backgrounds. The fourth factor

is psychoneurological i.e. the influence of stress and neurochemicals on the immune response. A common feature of these factors is their ability to affect gene expression.[27]

This study suggests that the diagnosis of an autoimmune disorder has to be made cautiously taking into consideration that, for all the above reasons, autoantibodies are present in low titres in the healthy population. The normal ranges for common autoantibodies that we have established for the Omani population may be a useful guide for diagnostic purposes [Table 3]. The establishment of normal cut-off values in clinically apparently healthy populations is important. Further structured epidemiological studies of autoantibodies throughout Oman should be undertaken.

CONCLUSION

This study clearly shows that autoantibodies do exist in normal healthy individuals of Oman. This fact should be taken into account when assessing the titres of antibodies considered to be of clinical significance in this population.

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