Evaluation of immunoglobulin isotypes and Rh factor among different human groups located in Aden district, republic of Yemen

Abstract
The aim of this study was to determine quantitatively the immunoglobulin Isotypes IgM, IgA, and IgG level as well as rheumatoid factor among Yemeni blood donors. Serum samples were collected from donors and utilized for nephelometric quantization of the immunoglobulin’s and RF. The donors were divided into three main groups
i. Smokers and non-smokers.
ii. Athletic and non athletic.
iii. Married and unmarried.
Results demonstrated that immunoglobulin level was found to be higher in married than unmarried, and in athletic than non athletic, whereas the level was lower in smokers than non smokers. RF was higher in married and in smokers than non-smokers, whereas lower in athletics than non athletics. This study suggests that implementation of these finding may attribute significantly to healthy and non-healthy human to establish national normal ranges in Aden district towards differential diagnosis based on all immunological and biochemical.

Keywords: Rh, IgG, IgM, IgA, Yemen, Aden, normal levels

Introduction
Immunological, biochemical national and international values are essential for herd immunity and scientific purposes. Rheumatoid factor (RF) is an antibody acting agent coordinated against the Fc portion of the IgG molecule.1 RF is found in 67-85% of patients, depending on the type of assay used. Rheumatoid factor (RF) is found in a high proportion of RA patients, it is additionally known to be show in non-rheumatoid patients.2 RF in rheumatic diseases has been shown to have considerable immunological / heterogeneity. Moreover, the regular IgM RF, both IgA RF and IgG RF have been almost exclusively recognized in patients with RA. In 2004 Kohayashi et al., found that a few patients with asthma have positive tests for rheumatoid factor (RF) which is recognized in systemic hyper-immune reactive disease for example, rheumatoid arthritis. The IgA-RF isotype has been identified to be associated with serious illness with erosions. The Isotypes groups of RFs are among the only auto antibodies obviously appeared to be included in disease pathogenesis.3 Xu et al., concluded that the IgM-RF may have high a significant role in the differential diagnosis RA. However, early study performed by Frangione et al., has been executed in UK and the results were found to be that Nonreactive 0-391U/ml, weakly responsive 40-79 IU/ml, Reactive>80 IU/ml. Hayahara et al., found that with the developing of the Japanese populace, an expansion in the number of elderly patients with rheumatoid arthritis (RA) has been noted as of late. The aim of the current study will be directed to evaluate the normal range of Rh Factor (RF) and immunoglobulin’s (Igs) in adult male serum in Aden locale, Yemen Republic.

Materials and methods
Subjects
Three hundred and fifty two blood samples were collected for four months from April to August 2013 from blood bank donors attending at the Ajamhuria Aden Hospital, Aden, and Republic of Yemen. The donors were healthy men between the ages 18-52. They don’t have any acute or chronic diseases. Candidates’ consent form with complete information requested including the name, age, marital status, blood pressure, pulse, Hb, temperature as well as donors life style such as smoking and exercise activities were obtained.

Ethical approval for this study was obtained from the Research Ethics Committee, the University of Aden, Aden, and Republic of Yemen. All of the subjects involved in the study gave signed informed consent before participation as general routine protocols for both blood donation and research studies.

Collection of blood samples
A 5ml of blood was taken from healthy individual in 4 test tubes of which; 1 EDTA sample for CBC examination by Hematology analyzer (sysmex machine). Whereas the other three plane tubes of were processed to separate the blood serum by centrifugation at 4000 rpm for 3 min and sent to chemistry lab (for liver function test (ALT, AST and ALP) and kidney function test (CREA and BUN) and serology lab for HIV-I, HIV-II, HBs Ag, Syphilis, Malaria, HTLV-I and HTLV-II screening tests using ELISA, as well as for immunoglobulin tests and rheumatoid factor using prospect machine.
Immunoglobulin and RF test

Anti-human IgM, IgG and IgA fluorescent marked are reagents probes of Igs tests and RF used in the Prospect machine (Dade Behring company). Nephelometric is the most commonly used estimation guideline for the immunochemical determination of protein. In this technique, antigen-antibody complexes are measured by scattered light.

Statistical analysis

All statistical tests were performed using SPSS (Apache Software Foundation, Wilmington, DE, version 12.0 for Windows). Data were analyzed using two-tailed t-tests to describe the normal range of serum proteins in different age of individuals.

Results

All collected (352) healthy men were included in this study. They didn’t have history of acute or chronic disease. The samples were classified into 5 groups between 18 and 52. The highest rate was 48.6% in group 20-29, whereas the lowest rate was 1.7 % in 50 and more (Table 1).

Table 1 Classification of samples on age base

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 or Younger</td>
<td>8</td>
<td>2.30%</td>
</tr>
<tr>
<td>20-29</td>
<td>171</td>
<td>48.60%</td>
</tr>
<tr>
<td>30-39</td>
<td>73</td>
<td>20.70%</td>
</tr>
<tr>
<td>40-49</td>
<td>94</td>
<td>26.70%</td>
</tr>
<tr>
<td>50 and More</td>
<td>6</td>
<td>1.70%</td>
</tr>
<tr>
<td>Total</td>
<td>352</td>
<td>100%</td>
</tr>
</tbody>
</table>

The Immunoglobulin quantization and Rheumatoid Factor test was performed on 285 negative samples. 67 samples were excluded as positive results. However, 207 samples were divided into three groups.

i. Marriages and singles.
ii. Smokers and non smokers.
iii. Athletic and non athletic.

On other hand, exclusion criteria was patients with HIV-I, II, HBsAg, HCVAb, Syphilis, Malaria, HTLV-I, II. Individual information was analysed on the basis which have been used to characterize the variation between normal individuals in Aden city. Table 2 shows the levels of fluctuation was used to compare the immunoglobulin’s and RF mean value between each subject’s of marriages & singles, smokers & non smokers, athletic and non athletic.

Table 2 Illustrate the immunoglobulin’s iso types in association with RF

<table>
<thead>
<tr>
<th></th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
<th>RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>Valid</td>
<td>285</td>
<td>285</td>
<td>285</td>
</tr>
<tr>
<td></td>
<td>Missing</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>1.59+</td>
<td>12.30+</td>
<td>0.201</td>
<td>2.10+</td>
</tr>
</tbody>
</table>

Assessment of the immunoglobulin Isotypes IgM, IgG and IgA levels, showed an increase in the group of marriages and athletics in contrast with single and non-athletic groups. Interestingly, there was a reduction in the levels of such immunoglobulin in the in the group of smokers when compared with non-smokers as appeared in Figure 1A-Figure 1C). For RF level, was found to be increase among marriages compared to singles as well among smokers compared to non smokers. Conversely, there were decline levels in the mean of athletics compared to non athletics as shown in Figure 2.
Discussion

This study was taken from 352 healthy male individual who are asymptomatic with no history of acute or chronic disease. The goal of this study was to evaluate the normal range of the serum concentration of Rheumatoid Factor and Immunoglobulin Isotypes in Aden city. During the investigated the value of normal range of IgG was found to be within the range between 6.63-16.71g/L, which is proportionally close to the international normal range (7.0-16.0g/L). As to the IgA the outcome was found to be 0.70-4.15g/L, and the international normal range is 0.7-4.0g/L. Whereas, the level of IgM was found to be 0.39-2.15g/L, in contrast of that of the international normal range (0.4-2.3g/L). There was no connection was shown between age and the level of immunoglobulin, which may explain that in adult level of immunoglobulin’s IgG, IgM and IgA isotypes were normally reached and maintained after ages 10years. Therefore no critical variations were found in immunoglobulin levels which could be credited to ages.

There was no relationship might have been exhibit between age and immunoglobulin concentrations, expound that the in adult level of immunoglobulin’s IgG, IgM and IgA isotypes were normally reached and maintained after ages 10years. Therefore, no consequential differences were found in immunoglobulin concentrations which could be attributed to ages. Our results were in total agreement when comparing will an additional investigation carried out by Buckley RH. In such study, Buckley revealed a statistical analysis where the serum immunoglobulin from the stage of infancy to adulthood, are displayed as geometric mean values (mg/100ml) showed that a consequential relationship between age and both IgG and IgA concentrations up to ages 6 and 7years.

Moreover, the results obtained from the smoker and non-smoker groups demonstrate that the IgG and IgA levels were fundamentally lower in the smokers than in the non-smokers whereas the IgE level was found to be higher in the smokers. Despite the IgM levels were likewise lower yet with the numbers considered not significant the IgD levels were found to be unaltered.

Furthermore, our results were in concurrence with other work of, in which the IgG and IgM levels in the serum were fundamentally lower in smokers in contrast with non-smokers (P<0.002). Despite the fact that IgA levels were low in smokers, it was not noteworthy. Antibodies were found in 14(93.3%) of 41 smokers (P<0.001), and the titers were found to be significant in nonsmokers. The serum IgG and IgA levels were higher in nonsmokers than in smokers, and the variation in that was found to be significant (F-test, P<0.05). Although the IgM level was additionally higher in nonsmokers than in smokers, yet this distinction was significant. Likewise the immunoglobulin levels in the athletes group were found to be higher than in the non-athletes American football players, the incidence of URTI was increased during intense training. This results were in total agreement with previous studies specially highly active athletes.

This increase is probably the result of contributions from extra vascular protein pools and an increased lymph flow, whereas in the resting state, immune function appears to be almost similar in athletes and non-athletes. While athletes at a periods of very heavy training, they have a dysfunction in the immune system, but it does not put them in danger of serious illness, but it increase their risk of picking up common infections such as URTI, or Influenza.

In regarding RF, the results demonstrated that the athletic group showed a significant finding in contrast with all other groups. This may explain that the incidence rate of the immune response to come in contact and interact with self-antigen may be declined to a level that gives other mechanisms to eliminate auto reactive B lymphocytes. This statement is in concurrence with a study performed by Cassidy et al., as stated that athletes at a periods of very heavy training, they have a dysfunction in the immune system, but it does not put them in danger of serious illness, but it increase their risk of picking up common infections such as URTI, or Influenza.

In conclusion, the present study has demonstrated that the range of the RF was found to be 9.23-18.70IU/ml (data not shown), in comparison with the international standardization range of 0-39IU/ml. The general outcome from this study showed that no significant correlation between the levels of immunoglobulin and age, however it has been found to be highly correlated among non-smoker and married group. In addition to that this study demonstrated that immunoglobulin levels in non-athletes are lower than in athletes but higher among smokers and married which may predict more likely to have an elevation of RF than non-smokers and single.

Acknowledgments
None.

Conflicts of interest
Author declares that there is no conflicts of interest.

References


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