

## ORIGINAL ARTICLE

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### Selective Immunoglobulin A Deficiency in Iranian Blood Donors: Prevalence, Laboratory and Clinical Findings

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#### ABSTRACT

Selective deficiency of immunoglobulin A (IgA) is the most frequent primary hypogammaglobulinemia. As some IgA-deficient patients have IgA antibodies in their plasma which may cause anaphylactic reactions, blood centers usually maintain a list of IgA-deficient blood donors to prepare compatible blood components.

In this study we determined the incidence of selective IgA deficiency (SIgAD) in normal adult Iranian population. 13022 normal Iranian blood donors were included in this study. The assay which we used was adapted to the manual pipetting system and ELISA reader was used for screening. Other classes of immunoglobulins (G, M), as well as secretory IgA and IgG subclasses were tested in IgA deficient cases by ELISA. SPSS was used for statistical analysis.

Among 13022 studied cases, 11608 blood donors were males (89.14%) and 1414 were females (10.86%). Their mean ( $\pm$ SD) age and weight were  $38.5 \pm 11$  years and  $82 \pm 12$  Kg respectively. Twenty of the screened samples were found by means of ELISA to be IgA-deficient (less than 5mg/dl), (frequency; 1:651). The data could indicate a compensation for IgA deficiency by serum IgM in one of our IgA deficient cases (Patient 5). We observed a correlation between IgG3 and serum IgA in deficient cases ( $r=0.498$ ,  $P=0.025$ ). Our results indicate that in present study the prevalence of S IgA D is in agreement with data from other Caucasians populations (from 1:300 to 1:700).

In conclusion, Selective IgA Deficiency could be almost asymptomatic in most cases in general population. Our study suggests that; due to high frequency of IgA deficiency in Iran, it seems necessary to measure IgA levels for every blood donor and blood recipient to find IgA deficient cases.

**Keywords:** Blood donor; IgA deficiency; Iran

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## INTRODUCTION

Selective IgA deficiency (sIgA-D) is the most common primary immunodeficiency disorder<sup>1,2</sup> with a frequency range between 1:163 reported in a normal Spanish population<sup>3</sup> and 1:18,500 in Japanese blood donors.<sup>4</sup> The prevalence of IgA deficiency varies from 1:223 to 1:1000 in community studies and from 1:400 to 1:3000 in normal blood donors.<sup>5</sup>

A number of IgA-deficient patients have anti-IgA in their plasma, which may cause anaphylactic reactions after transfusion of IgA-containing blood products.<sup>6,7</sup> Such individuals should be transfused blood and components deficient in IgA, which can be obtained from known IgA deficient blood donors or using blood component which its IgA has been physically removed.<sup>8</sup> Since transfusing blood components obtained from IgA deficient donors seems to be a better alternative for these cases, every Blood Transfusion Center should have a donor registry of IgA deficient donors depending on the prevalence of IgA deficiency in the population to prepare compatible blood components for these patients.

IgA deficient individuals are usually asymptomatic and thus are rarely diagnosed.<sup>9</sup> It is important to know its prevalence as it helps the blood banks to assess the need to have IgA deficient blood components.

As the prevalence of IgA deficiency in Iran so far is unknown, in the present study IgA deficiency is evaluated in blood donors of Iranian Blood Transfusion Organization.

## MATERIALS AND METHODS

During 18 months of study (Mar. 2005- Sep. 2006), 13022 healthy voluntary blood donors in Iranian Blood Transfusion Center in Tehran were included in this study. The donors selection were based on a detailed medical history. Normal physical examination performed by a General Physician. Furthermore laboratory examinations were done in the Blood Transfusion Center for viral infections including HIV, HBV and HCV which were normal in all selected cases.

The control cases in this study were age-sex-matched with each IgA deficient subject. After health confirmation by Blood Transfusion Center criteria, Immunologic tests including IgA, IgG, IgM, IgG subclasses and secretory IgA were done for this group concurrently as done as for IgA deficient cases.

## Serum Collection and Analysis

Serum samples which Iranian Blood Transfusion Organization approved according to their criteria were collected after blood donation and stored at -20°C until analysis. IgA deficiency was detected using an enzyme linked immunosorbent assay (ELISA) which was developed in immunology laboratory of Immunology, Asthma and Allergy research Institute (IAARI). The IgA assay was done in microwell plates coated with monoclonal mouse anti-human IgA antibody as follows:

Maxisorb micro titer plates (NUNC, Denmark) were coated with 100 µL per well of a 1/4,000 dilution (2.5µg per mL) of monoclonal mouse anti-human IgA (SIGMA, USA) in the coating buffer, sealed and incubated overnight at 2-8°C in wet chambers. After Three washes with washing solution (PBS with 0.05 percent Tween 20) by automatic micro plate washer (Bio Rad Laboratories Inc., CA, USA), plates were decanted, filled with 225 µL of blocking solution (5% BSA in PBS) per well and kept for 2 hours at 37°C to block any remaining binding sites. Thereafter, plates were decanted, washed for three times, sealed with plastic films and stored in humid chambers at 2-8°C for one week without loss of activity.

Wells of coated micro plates were washed three times before sample addition. One hundred micro liters of calibrators (DAKO, Denmark), donor samples (serum), control serum (SERO, Norway) and blank (diluent) were pipetted directly into the specified wells. Calibrators (concentrations of IgA were determined by diluting the human immunoglobulin calibrator in the assay diluent from zero up to 220 mg/dL) were always tested in duplicates. Plates were sealed and incubated for 90 minutes at 37°C. After being washed, 100µL of horseradish peroxidase-conjugated goat anti-human IgA (SIGMA, USA) was added to each well. Following 60 minutes of incubation at 37°C, plates were washed and bound IgA was detected by the addition of 100µL of chromogen (10mg of TMB/DMSO in 25 ml of 0.15 M sodium-citrate buffer, containing 5 µl of 30% v/v H<sub>2</sub>O<sub>2</sub>). The enzymatic reaction was stopped by the addition of 50 µl 20% H<sub>2</sub>SO<sub>4</sub> and the absorbance was read at 450nm (against 630nm as the reference wavelength) within 30 minutes. Absorbance values were corrected automatically for the mean absorbance of blank which had to be less than 0.100. Different dilutions of standard (containing known concentrations of IgA) were run in each plate to plot a standard curve.

Serum IgA concentrations were calculated using this curve. The sensitivity of the assay was 0.035 mg/dL. Selective IgA deficiency was defined as the IgA value less than 5 mg/dl,<sup>10</sup> in the presence of normal serum IgG and IgM levels. These Immunoglobulins were assayed by Binding Site Nephelometry kit (MININEPH, Birmingham, UK) using the identical analyzer unit. IgA-deficient samples were retested in duplicate on another plate to confirm the initial result. These cases were also tested for IgG subclasses (IgG1, IgG2, IgG3 and IgG4) by ELISA (Sanquin, Netherlands) according to the manufacturer's instruction and results were compared to normal ranges.<sup>11</sup>

Secretory IgA was also been detected for the deficient cases using an enzyme linked immunosorbent assay which the detail follows.

#### Saliva Collection and Analysis

The saliva samples were collected following mouth washing and taking prohibitor for 20 minutes. The saliva samples were then stored at -70°C until analyzed.<sup>12</sup> At the test time they were thawed and centrifuged for 10 min at 3200g to remove cells and debris. The supernatant of each sample was diluted in phosphate buffer saline containing 0.05% Tween 20. Diluted saliva samples (1/100 dilution) were added in a volume of 100µl into the micro titer wells of prepared ELISA plates as mentioned before. In these plates serial dilutions for the IgA standard had lower concentrations (from zero up to 16 mg/dL) for plotting the appropriate standard curve.

#### Statistical Analysis

SPSS 11.5 software was used for statistical analysis. Pearson correlation test was used to calculate the correlation between laboratory findings of IgA deficient cases.  $P < 0.05$  was considered statistically significant.

## RESULTS

Among 13022 cases, 11608 volunteer blood donors were males (89.14%). Their mean ( $\pm$ SD) age and weight were  $38.5 \pm 11$  years and  $82 \pm 12$  Kg. A total of 20 patients (Male: Female = 18:2) was detected as IgA deficient among 13022 cases (frequency; 1:651). The age of deficient cases varied from 21 to 41 yr. The mean value of IgA in each group is mentioned in Table 1.

Table 2 enlisted absolute levels of immunoglobulins in deficient cases. Serum IgA was absent in six cases. We observed a correlation between IgG3 and serum IgA in IgA deficient cases ( $r=0.498$ ,  $P=0.025$ ) compared to control group ( $r=0.359$ ,  $P=0.12$ ).

Confirmed IgA-deficient blood donors were informed about their problem by an immunologist and were provided with additional information. These donors received a card stating that they were IgA-deficient and were warned for necessary precautions.

The most common presenting clinical symptom in IgA deficient cases was recurrent infections (30%). Upper respiratory tract infection was the most predicted site of infection as 5 patients (20%) had recurrent sino-pulmonary infections while gastrointestinal tract problems had suffered two patients (10%); one hospitalized case following acute gastroenteritis in childhood and another one with recurrent diarrhea. Only one out of twenty IgA deficient patients have had autoimmune problem (5%); which presented juvenile arthritis since 3 years old.

## DISCUSSION

The results clearly showed that the incidence of Selective IgA Deficiency (S-IgA-Def.) in Iranian blood donors (frequency; 1:651) is considerable in this country compared with other studies around the world

**Table 1. Mean values of serum and saliva IgA in selective IgA deficient cases, normal cases and control Group.**

Group	No.	Serum IgA (mg/dl)	Saliva IgA (mg/dl)
Selective IgA Deficient cases	20	$0.356 \pm 0.295$	$0.225 \pm 0.076$
Normal cases	13002	$87.7 \pm 140.7$	ND*
Control group	20	$92.5 \pm 123.8$	$1.86 \pm 3.46$

\* ND=Not Done

Table 2. Absolute levels of immunoglobulins in IgA deficient cases.

Patient	Saliva	Serum immunoglobulins (mg/dL)						
	IgA	IgA	IgG	IgM	IgG1	IgG2	IgG3	IgG4
1	.0	.0	2076	51.0	967	774	115	75
2	.0	.0	1487	80.0	870	462	106	85
3	ND	.0	1900	104.0	1328	324	75	104
4	.0	.0	1585	62.7	840	482	146	101
5	.1	.0	1945	489.0	1263	478	35	64
6	.0	.0	1755	48.1	945	662	92	82
7	.0	.1	2360	75.0	1403	658	128	69
8	.0	.1	1384	56.0	612	454	172	101
9	.0	.1	2392	60.0	876	1143	100	148
10	.0	.1	1825	95.6	1245	306	62	102
11	.0	.1	1783	171.1	1113	480	91	74
12	.0	.2	2468	80.0	1040	1290	132	62
13	.0	.2	2659	75.4	1679	627	110	108
14	.0	.4	2069	84.0	1068	853	129	120
15	.0	.4	1498	231.3	981	320	88	85
16	ND	.6	1402	72.0	865	439	157	58
17	.3	.7	1461	213.4	1002	302	98	58
18	.0	.8	2247	30.0	1455	520	117	84
19	ND	1.0	2740	134.0	1345	1024	178	166
20	.9	1.1	2785	92	1546	898	159	153
<b>Normal Range</b>	<b>1.6-5.32<sup>*</sup></b>	<b>72-375<sup>*</sup></b>	<b>636-1837<sup>*</sup></b>	<b>39-283<sup>*</sup></b>	<b>280-1020<sup>+</sup></b>	<b>60-790<sup>+</sup></b>	<b>14-240<sup>+</sup></b>	<b>11-330<sup>+</sup></b>

<sup>\*</sup>Mean of control group; <sup>\*</sup>Reference (21) for serum IgA, IgG and IgM.; <sup>+</sup>Reference (11) for IgG subclasses.

(1:163– 1:18500).<sup>3-5</sup> This result is in contrast with prevalence of IgA deficiency in countries such as Japan that has been reported as low as 1:18500.<sup>4</sup> The difference in prevalence may be due to different ethnicities in these countries but our result is in agreement with data from other Caucasians' populations, the prevalence in Caucasians varies from 1:300 to 1:700.<sup>13</sup>

IgA deficiency is associated occasionally with IgG subclass deficiency, a combination that leads to severe bacterial infections.<sup>14,15</sup> Previous studies had been suggested that IgA deficiency is associated with those of the IgG subclasses, especially of IgG2, IgG3 and IgG4.<sup>16,17</sup> Hammarström *et al.* reported a simultaneous change in the IgG subclass pattern with a lack of specific anti-polysaccharide antibodies of the IgG2 subclass.<sup>18</sup> In our present study we observed a correlation between IgG3 and serum IgA concentrations in IgA deficient cases. Our result is in agreement with data from Kutukculer *et al.* where

among 87 studied patients, IgG3 subclass deficiency was the most frequent defect combined with IgA deficiency.<sup>19</sup> IgG3 is mostly constituted of antibodies directed against viral antigens.<sup>20</sup> It must be mentioned that in Kutukculer's study IgG3 deficiency combined with IgA deficiency was found in children, but our study population included adult cases, furthermore, we did not find any IgG3 deficient patient.

The data could indicate a compensation for IgA deficiency by serum IgM in one of IgA deficient cases (Patient 5).

Our results indicated that ELISA is a good quantitative screening test for IgA deficiency also the instruments are installed in most of the laboratories that can be adapted by micro well plates in bulk.

IgA deficiency is a heterogeneous disease with variable presentations ranging from asymptomatic to recurrent sinopulmonary infections and/or gastrointestinal tract infections. Since all of the samples derived from volunteers referring to Iranian Blood

## Prevalence of Selective IgA Deficiency in Iran

Transfusion Center and the volunteer blood donors mostly felt healthy, thus S-IgA-Deficiency could almost be asymptomatic in a lot of cases in general population. However due to exclusion of symptomatic IgA deficient patients by Blood Transfusion Center or for the reason that these symptomatic cases may not come as a volunteer for blood donation, our subjects were limited to almost asymptomatic IgA deficient cases.

According to our findings; due to high frequency of IgA deficiency in Iran, it seems necessary to measure IgA level for every blood donor as well as blood recipient to find IgA deficient cases in order to minimize transfusion problems. It is recommended that every Blood Transfusion Center should provide and expand a blood donor bank for IgA deficient donors, hence prepare compatible blood products for these patients.

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