Low prevalence of IgA deficiency in north Indian population

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Background & objectives: IgA deficient patients are at risk of severe anaphylactic reaction on being transfused blood and blood products, and its prevalence varies in different parts of the world. No data are available from India. We did a blood donor survey to look for prevalence of IgA deficiency in north India.

Methods: A sensitive enzyme linked immunosorbent assay developed in-house was used to detect IgA deficiency in a total of 3818 blood donors. Complete IgA deficiency was defined as value less than 5 mg/dl whereas partial IgA deficiency was defined as value between 5-30 mg/dl.

Results: Of the 3818 blood donors screened, 3640 (95.3%) were males with a mean age of 31.2 yr. No donor was found to have complete IgA deficiency; however, 257 (6.7%) had partial IgA deficiency.

Interpretation & conclusion: Our study shows that IgA deficiency is rare in north India.

Key words Immunodeficiency - immunoglobulin - transfusion reactions

Non haemolytic transfusion reaction is the most common complication of blood transfusion. Among this, febrile non haemolytic transfusion reactions (FNHTR) are commonest, whereas allergic reactions rarely occur. Some of the anaphylactic/anaphylactoid reactions are caused by anti-immunoglobulin A antibodies (anti-IgA) in IgA deficient recipients.

Selective IgA deficiency (serum IgA < 0.5 mg/dl) is the most common primary immunodeficiency disorder. IgA deficient individuals can get immunized and develop anti IgA antibodies and subsequent transfusion of blood products containing IgA protein can lead to life threatening anaphylactic reactions. Antibodies to IgA are present in 44 percent of IgA deficient individuals. Such individuals should be transfused blood and components deficient in IgA, which can be obtained from known IgA deficient blood donors or by physically removing IgA from blood components, such as by washing red cell components with saline. Every blood transfusion center should therefore have a donor registry of IgA deficient individuals.
deficient donors, depending on the prevalence of IgA deficiency in the population.

Several screening tests for IgA deficiency are available like, passive haemagglutination inhibition assay (PHAIA)\(^5\), membrane enzyme immune assay (EIA), enzyme linked immunosorbent assay (ELISA)\(^6\), solid phase red cell adherence assay (SPRCA)\(^7\), radial immunodiffusion (RID)\(^8\) and rate nephelometry\(^9,10\), each having its own merits and demerits.

The prevalence of selective IgA deficiency varies from 1:320 in US\(^11\) to 1:18,500 in Japan\(^12\). The prevalence of IgA deficiency in India is not known. IgA deficient individuals are usually asymptomatic and thus are rarely diagnosed. It is important to know its prevalence as it helps the blood banks to assess the need to have IgA deficient donor pool. The present study was therefore carried out to screen normal healthy blood donors to know prevalence of IgA deficiency in north Indian population.

**Material & Methods**

A total of 3,818 non remunerated voluntary or relative blood donors donating blood at Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India during March to November 2003 were included in the study. The donors were selected in strict confidence based on a detailed medical history and a brief examination. Further, criteria laid down by the Drugs & Cosmetics Act of India, 1945\(^13\) for donor selection were strictly followed. Serum samples were collected during blood donation and stored at -70°C till analysis.

IgA deficiency was detected using an in-house enzyme linked immunosorbent assay (ELISA) following method described by Sanz et al\(^6\). Briefly, microtitre plates (Nunc, Germany) was coated with 50 μl of rabbit anti-human IgA (Dakopatts, Denmark) per well at a concentration of 5 μg/ml in carbonate-bicarbonate buffer. The plate was incubated at 37°C for 1 h and then overnight at 4°C. Next day, after washing the plate with phosphate buffer saline (PBS) (pH 7.2, 150 mmol), 250 μl of 4 per cent bovine serum albumin (BSA) (Sigma Chemicals, USA) in PBS was added to each well as blocking agent and the plate was incubated at 37°C for 2 h. The plate was again washed with PBS and 50 μl of test serum diluted 1:5000 in PBS- 1 per cent BSA was added to wells in the plate in duplicate and incubated at 37°C for 90 min. After washing the plates with PBS-Tween 20 (Merck, Germany), 50 μl of diluted (1:6000 in PBS) horseradish peroxidase conjugated rabbit anti-human IgA (Dakopatts, Denmark) was added to each well and the plate was incubated for 90 min. After washing, 50 μl of substrate (O-phenylenediamine dichloride (OPD, Sigma Chemicals and H₂O₂ in phosphate-citrate buffer) was added. The colour was then read at 492 nm in an ELISA reader (Tecan, Spectra, Germany) after adding 25 μl of stop solution (0.1N H₂SO₄). Standard curve was generated using serum containing known concentrations of IgA. Different dilutions of the standard were run in each plate to get a standard curve. Test serum concentration was calculated using this curve. The sensitivity of the assay was 0.05 mg/dl.

Complete IgA deficiency was defined as IgA value less than 5 mg/dl whereas partial IgA deficiency was defined as IgA value between 5-30 mg/dl\(^10\).

**Results & Discussion**

Among the 3818 voluntary blood donors, 3640 were males (95.3%). Their mean age was 31.2 ± 4.2 yr with range varying from 18 to 60 yr. None of the donors was found to be IgA deficient. However, 257 (6.7%) had partial IgA deficiency, and of these 18 had values between 5-10 mg/dl. No relationship of partial IgA deficiency and age or gender of the donor was found.

ELISA was first used by Sanz et al\(^6\) in 1999 to screen blood donors for IgA deficiency. It had a sensitivity of 0.01 mg/dl. Using the same principle, an in-house ELISA was developed with a sensitivity of 0.05 mg/dl. The sensitivity was a little lower as compared to the original method but was adequate for detecting IgA deficiency (>5 mg/dl). Our results indicated that ELISA was a good screening test for IgA deficiency. It offers the advantages of being quantitative and sensitive; the instruments are already
installed in most of the blood banks. Moreover, ELISA can be adapted for automation and made user friendly by preparing microwell plates in bulk.

Our results are in agreement with data from other Asian countries, such as Japan\textsuperscript{12} and China\textsuperscript{14}, where the prevalence of IgA deficiency has been reported to be as low as 1:18,500 and 0:5,300, respectively. The prevalence of IgA deficiency is more common in Caucasians and people of European decent. In a study from USA\textsuperscript{11}, the prevalence of IgA deficiency in blood donors is reported to be as high as 1:320, while in a study from Europe\textsuperscript{15}, the prevalence in Caucasians varies from 1:300 to 1:700. Another reason for very low prevalence of IgA deficiency in our blood donors may be due to the selection bias because symptomatic IgA deficient individuals would have been deferred as blood donors.

The definition of IgA deficiency varies considerably in the literature from 1 mg/dl to 10 mg/dl and may reflect the sensitivity of the techniques used (Table). For example, two different studies\textsuperscript{9,6} defined IgA deficiency as <10 mg/dl and <5 mg/dl, respectively. Koistinen\textsuperscript{16} demonstrated an inverse relationship between sensitivity of the technique and frequency of the IgA deficiency. For instance, two studies from the same country using two different methods have reported different prevalence; thus, one study that used nephelometry\textsuperscript{9} for screening reported the prevalence as 1:163, whereas the other study\textsuperscript{6} reported the prevalence as 1:655 using ELISA for screening. However, our data suggested that using a cut-off of 5 mg/dl, the prevalence of IgA deficiency in our country was very low; if a less stringent definition of 10 mg/dl is used then the prevalence would be 1:212.

As per Litzman \textit{et al}\textsuperscript{10}, individuals with serum IgA levels from 5 to 30 mg/dl are classified as being partially deficient for IgA. In our study, 257 of the 3818 donors screened (6.7\%) had serum IgA levels ranging from 5 to 30 mg/dl. However, these partially deficient individuals are of no clinical significance, as they do not form anti-IgA antibodies, which are responsible for anaphylactic type of transfusion reactions. Anti-IgA antibodies have only been reported in individuals with serum IgA <5 mg/dl or in whom IgA subclass (IgA\textsubscript{1}, or IgA\textsubscript{2}) is deficient\textsuperscript{21}.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Samples tested (N)</th>
<th>Method</th>
<th>Sensitivity (mg/dl)</th>
<th>Frequency of IgA deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vyas \textit{et al} (1975)\textsuperscript{7}</td>
<td>73569</td>
<td>ID</td>
<td>&lt;2</td>
<td>1:650</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PHI</td>
<td>&lt;0.05</td>
<td>1:886</td>
</tr>
<tr>
<td>Koistinen (1975)\textsuperscript{16}</td>
<td>64588</td>
<td>ID</td>
<td>&lt;2</td>
<td>1:396</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PHI</td>
<td>&lt;0.05</td>
<td>1:507</td>
</tr>
<tr>
<td>Ropars \textit{et al} (1982)\textsuperscript{19}</td>
<td>10800</td>
<td>Groupamatic</td>
<td>&lt;2</td>
<td>1:1300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RIA</td>
<td>&lt;0.002</td>
<td>1:2500</td>
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<tr>
<td>Hunt \textit{et al} (1985)\textsuperscript{19}</td>
<td>41851</td>
<td>PHI+ELISA</td>
<td>&lt;0.02</td>
<td>1:721</td>
</tr>
<tr>
<td>Schuleenburg \textit{et al} (1991)\textsuperscript{7}</td>
<td>6117</td>
<td>SPRCA</td>
<td>&lt;1</td>
<td>1:340</td>
</tr>
<tr>
<td>Mohabir \textit{et al} (1995)\textsuperscript{20}</td>
<td>5723</td>
<td>Olympus PHI+ELISA</td>
<td>&lt;0.01</td>
<td>1:954</td>
</tr>
<tr>
<td>Present study (2004)</td>
<td>3818</td>
<td>ELISA</td>
<td>&lt;0.05</td>
<td>0:3818</td>
</tr>
</tbody>
</table>

ID, immunodiffusion; PHI, passive haemagglutination inhibition; Groupamatic, Kontron automated blood grouping machine using passive haemagglutination inhibition for IgA deficiency screening; RIA, radioimmunoassay; ELISA, enzyme linked immunosorbent assay; SPRCA, solid phase red cell adherence assay; Olympus-automated blood grouping machine using passive hemagglutination inhibition for IgA deficiency screening
In conclusion, our study suggests that IgA deficiency is not a clinically significant problem in north Indian blood donor population. Such studies need to be done in all parts of India with a much larger sample size to know the exact prevalence of IgA deficiency in India.

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