IMMUNOGLOBULIN A (IGA) LEVELS IN HEALTHY BLOOD DONORS IN YAOUNDE, CAMEROON

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ABSTRACT

Background and Objectives: IgA deficient patients are at risk of severe life-threatening anaphylactic reaction on being transfused blood or blood products. This study aimed at assessing the prevalence of IgA deficiency in blood donors in Yaounde, Cameroon.

Methods: Serum samples were collected from 670 apparently healthy blood donors. Enzyme linked Immunosorbent Assay (ELISA) was used to quantitate total IgA concentration.

Results: Of the 670 samples tested, 536 (80%) were from males and 134 (20%) from females. Age ranged from 17 to 58 with a mean of 28.3 ± 7years. There were no patients with IgA deficiency (IgA <5mg/dL); 11 persons (1.6%) with partial IgA deficiency (5-30mg/dL); 535 (79.9%) with normal IgA (31-450mg/dL) and 124 (18.5%) with greater than normal IgA levels. The concentration of IgA varied by sex, with females having significantly higher levels than males (p=0.002). The mean IgA
concentration generally increased with increasing age, p=0.001).

Conclusion: This study suggests that IgA deficiencies are rare in the study group and may not be a major clinical concern in our setting.

Key words: Immunoglobulin A levels; Blood donors; Serum samples; IgA deficiency; Cameroon.

RESUME

Introduction : Les patients déficients en IgA sont à risque de réactions anaphylactiques sévères suite à une transfusion sanguine ou à des dérivés sanguins. Cette étude a pour but d'évaluer la prévalence du déficit en IgA chez les donneurs de sang à Yaoundé, Cameroun

Méthodes : 670 sérums ont été recueillis chez les donneurs de sang apparemment en santé. Le test ELISA (Enzyme Linked ImmunoSorbent Assay) a été utilisé pour quantifier les IgA totales.

Résultats : Des 670 échantillons testés, 536 (80%) provenaient de sujets de sexe masculin et 134 (20%) de sujets de sexe féminin. L'âge variait de 17 à 58 ans avec une moyenne de 28,3±7ans. Aucun patient ne présentait une déficience en IgA (IgA<5mg/dl), 11 sujets (1,6%) présentaient une déficience partielle en IgA (5-30mg/dl) ; 535 (79,9%) avaient une concentration en IgA normale

(31-450mg/dl) et 124 (18,5%) avaient une concentration en IgA supérieure à la normale. La concentration en IgA variait avec le sexe, les sujets de sexe féminin présentant une concentration en IgA supérieure à celle des sujets de sexe masculin (p=0,002). La concentration moyenne en IgA augmentait avec l'âge (p=0,001).

Conclusion : Cette étude suggère que les déficiences en IgA sont rares dans la population d'étude et ne pourraient être un problème clinique majeur dans notre contexte.

Mots clés : Concentration en IgA, donneurs de sang, déficience en IgA

INTRODUCTION

Selective Immunoglobulin A (IgA) deficiency (serum IgA <5mg/dl) is the most common primary immunodeficiency disorder [1, 2]. IgA deficient individuals can get immunized or exposed, after which they develop anti-IgA antibodies. Subsequent transfusion of blood products containing IgA antiboidies can lead to serious transfusion reactions that could be life-threatening with generalized symptoms [3, 4]. Antibodies to IgA are present in 44 per cent of IgA deficient individuals and these antibodies are usually of the Immunoglobulin G (IgG) class, less commonly Immunoglobulin M (IgM) and rarely Immunoglobulin E (IgE) [4]. Such individuals should be transfused blood and components deficient in IgA, which can be obtained from known IgA deficient blood donors or by washing red cell components with saline to physically remove IgA from blood components. Blood transfusion centers may therefore need to have a registry of IgA deficient donors, depending on the prevalence of IgA deficiency in the population.

The prevalence of selective IgA deficiency varies greatly depending on the region or race [5]. The prevalence of IgA deficiency in Cameroon is however not known. Since IgA deficient individuals are usually asymptomatic and are rarely diagnosed, quantifying the prevalence of IgA deficiency would provide data for blood banks to assess the need to have a pool of IgA deficient donors. This study was carried out to screen a group of normal healthy blood donors in Yaounde to determine the prevalence of IgA deficiency.

MATERIALS AND METHODS

Patients

We recruited 670 consecutive and apparently healthy blood donors in the blood bank of the Yaounde Central Hospital. Participants were recruited in this study after they underwent regular pre-donation activities in the blood bank, including measurement of blood pressure, weight, physical examination and medical history. All eligible blood donors who provided written consent and tested negative for Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) were included in the study. Pregnant women, breastfeeding mothers and women on their menses were excluded.

Preparation of Samples and Standards

Serum samples were obtained from 5mL of whole blood collected from each donor in a dry tube and allowed to clot. The samples were pre-diluted in the Sample Diluent (Tris Buffered Saline :TBS - 1 % Bovine Serum Albumin :BSA and 0.05% Tween 20) to 1:5 000. The following concentrations of the standard (from manufacturer) were prepared in the Sample Diluent: 500ng/mL, 250ng/mL, 125ng/mL, 62.5 ng/mL, 31.25 ng/mL, 15.625 ng/mL, 7.8 ng/mL. These concentrations were used to generate a standard curve from which the test serum IgA concentrations were read.

ELISA Assay

IgA concentrations were determined using a Human IgA Quantitation Enzyme Linked Immunosorbent Assay (ELISA) kit from Bethyl Laboratory, (USA). Microtitre plates (MTP) (Nunc (MaxiSorp), Germany), were coated with 100µL of Goat anti-human IgA-affinity purified (Bethyl Laboratories, USA) per well at a concentration of 0.01µg/ml in 0.05M carbonate-bicarbonate buffer, pH 9.6. The plates were incubated at room temperature (RT) for 60minutes and then washed three times with TBS (pH 8.0, 50mM), with 0.05% Tween 20 (Sigma Chemicals, USA). 200µL of 1 % bovine serum albumin (BSA) (Sigma Chemicals, USA) in TBS (pH 8.0, 50mM) was added to each well as blocking buffer and the plate was incubated at RT for 30minutes. After blocking the plates, they were again washed three times with TBS and 100µL of diluted test serum and standards were added to wells in the plate in duplicates and incubated at RT for 60 minutes. After washing the plates five times with TBS-Tween 20, 100µL of diluted (1:50000 in TBS) goat anti-Human IgA-Horseradish Peroxidase (HRP) Conjugate (Bethyl, USA) was added to each well and the plate was incubated for 60 minutes at RT. After five additional washes, 100µL of enzyme substrate (Tetra Methyl Benzidine, TMB-peroxidase substrate and peroxidase solution, Sigma Chemicals) was added. This was allowed to react for 30minutes and the reactio was then read at 450 nm in an ELISA reader (Tecan, Spectra, Germany). The optical density values corresponding to the standards were used to construct a standard curve from which test sera concentrations were read off.

Statistical Analysis

Data were analyzed using STATA version 9 (STATA Corp., College Station, Texas, USA). IgA concentrations were categorized as either 'IgA deficiency' (IgA <5mg/dL); 'partial IgA deficiency' (5-30mg/dL); 'normal IgA' (31-450mg/dL) or 'greater than normal IgA' levels. Fisher's exact tests were used to compare proportions of each IgA category by age and sex categories. Values of $p< 0.05$ were considered statistically significant.

RESULTS

Levels of IgA concentrations

There were 536 male and 134 female black African (Cameroonian) donors included in this study. Their ages ranged from 17 to 58 with a mean age of 28.3 ±

7years. Of these 670 donors, 11 (1.6%) had partial IgA deficiency (5-30mg/dL); 535 (79.9%) had normal IgA concentrations (31-450mg/dL); and 124 (18.5 %) had higher than normal IgA levels (>450mg/dL). None of the participants had IgA deficiency (<5mg/dL) as shown on Table 1.

IgA concentration significantly varied by age and sex (Table1). IgA levels tended to increase with age: only 14.5% of 392 participants aged 20-29 had levels > 450mg/dl versus 36.5% of the 74 participants aged ≥40. On the other hand, 2.3% of those aged 20-29 had a partial IgA deficiency versus none in those aged ≥ 40. While the proportion of females (1.5%) and males (1.7%) with partial IgA deficiencies were similar, females still had higher levels of IgA - 29.1% of females (versus 15.9% of males) had IgA levels ≥450mg/dl (Table 1).

DISCUSSION

The prevalence of IgA deficiency in Yaounde was very low (0 per 670) in this study using a cut-off of 5 mg/dL; if the less stringent definition of less than 10 mg/dL is used then the prevalence would have been 1 in 84. It may therefore not be absolutely necessary to routinely screen blood donors or recipients for IgA deficiency in the study population. The IgA levels in our study subjects, like in the study of Weber-Mzell *et al* in 2004, were dependent on the sex and age of the donors. Females in the group had significantly higher IgA levels than males. This was more evident in women between the ages 20 - 29 years. This high IgA concentration may be due to hormonal influence in these women of child bearing age as IgA is secreted in breast milk and helps in protecting the infants. The increase in IgA concentration with age is expected as it is responsible for immune surveillance on mucosal surfaces that are continually exposed to a variety of infectious and noxious agents, older persons probably having been exposed more.

Although no complete IgA deficiency was found, there were cases of partial IgA deficiency. As proposed by Litzman *et al*; in 2000, individuals with serum IgA levels from 5 to 30 mg/dL are classified as being partially deficient for IgA. In this study, 11 of the 670 donors screened (1.6%) had serum IgA levels ranging from 5 to 30 mg/dL. However, these partial deficiencies are of no clinical significance, as these individuals do not form anti-IgA antibodies, which are responsible for anaphylactic type transfusion reactions. Anti-IgA antibodies have only been reported in individuals with serum IgA <5 mg/dl or in whom IgA subclass (IgA1 or IgA2) is deficient [7].

As many as 124 (18.5%) of the donor population had hyperimmunoglobulin A. Reactions that occur may be proportional to the IgA antibody levels. On the other hand, there seems to be no particular serious risk to the hyper IgA donors. This high IgA concentration may however be important in the protection of the mucosal surfaces against pathogens such as in the respiratory mucosa, the gastrointestinal mucosa and the genitourinary mucosa.

Our results are in agreement with data from Asian countries, such as Japan [8] and China [9] where the prevalence of IgA deficiency has been reported to be as low as 1:18,500 and 0:5,300, respectively. Our results are also similar to data from American studies which show that the prevalence of IgA deficiency is very low in people of African origin [5]. The prevalence of IgA deficiency is more common in Caucasians and people of European decent but rare in Asians and people of the black race [10,11]. In a study from the USA the prevalence of IgA deficiency in blood donors was reported to be as high as 1:320 [12], while in a study from Europe the prevalence in Caucasians varied from 1:300 to 1:700 [13].

A relatively high proportion of the study population had very high levels of IgA. The possible reasons are racial or genetic factors and environmental factors [5]. The study population consisted of all blacks from the Cameroon. The study area is in the tropical region of Africa with many infectious and parasitic diseases, most of which are endemic. However, the effects of disease and infections on IgA levels are limited and not conclusive [5]. Malaria, for example, that is endemic in Yaounde may cause raised levels of IgA, but this has not been investigated. Other infections that may affect levels of IgA are intestinal parasites, tissue and blood microfilaria parasites and bacterial infections such as *Mycobacterium tuberculosis* which are more frequent in the region.

Another reason for this difference may be the difference in sensitivity of the method used in the analysis and laboratory conditions. The levels may also vary depending on the laboratory and the technique used. This is why each laboratory should establish its own normal ranges, and normal values established for each population. 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. For example, two different studies defined IgA deficiency as <10mg/dL and<5mg/dL, respectively [6,14]. These results indicate that the more sensitive the technique, the lesser the frequency of IgA deficiency. For instance, Pereira *et al*. in 1997 in Spain used nephelometry for screening and reported the prevalence as 1:163, whereas Litzman *et al.* in another study in 2000 reported the prevalence as 1:655 using ELISA for screening.

In conclusion, this study suggests that IgA deficiencies are rare in the study group and may not be clinically significant in our setting. Older age and female sex are associated with increased IgA levels. This study was carried out in healthy blood donors expected to be representative of the general population. However all participants were seen in an urban area. IgA levels in healthy participants in rural areas and patients in different pathologic conditions will still need to be assessed to offer a more complete picture.

Table 1: Distribution of IgA concentration by age and sex

	N	Category of IgA Concentration - N (%)				p-value*
		<5 mg/dL	**5-30 mg/dL**	**31-450 mg/dL**	**>450 mg/dL**	
Age						0.001
≤19	43	0 (0)	1 (2.3)	35 (81.4)	7 (16.3)	
20-29	392	0 (0)	9 (2.3)	326 (83.2)	57 (14.5)	
30-39	161	0 (0)	1 (0.6)	127 (78.9)	33 (20.5)	
≥40	74	0 (0)	0 (0)	47 (63.5)	27 (36.5)	
Sex						0.002
Females	134	0 (0)	2 (1.5)	93 (69.4)	39 (29.1)	
Males	536	0 (0)	9 (1.7)	442 (82.5)	85 (15.9)	
Total	670	0 (0)	11 (1.6)	535 (79.9)	124 (18.5)	

* Fisher's exact p-value

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