Measurement of IgA Level in Normal Human Tears by Enzyme-linked Immunosorbent Assay

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The advantages of the enzyme-linked immunosorbent assay (ELISA) are its sensitivity and its accuracy in detecting and measuring immunoglobulin classes. By ELISA, the tear IgA level was measured in 165 healthy persons. Tears were collected by a strong absorbent (Weck Cel+). This is the first report of a tear IgA level in normal Koreans measured by ELISA. The mean level was 60.5 ± 23.4 mg/dl. There was no statistically significant difference between the IgA level in the males (61.8 ± 23.2 mg/dl) and that of the females (58.4 ± 23.5 mg/dl)(p>0.05). The difference between the tear IgA levels in the various age groups was not significant (p>0.05).

Key words: enzyme-linked immunosorbent assay (ELISA), IgA, tears, normal.

INTRODUCTION

Human tear film consists of three layers—the lipid, aqueous and mucinous layers. The wetting of the cornea is most important function of tears. Besides this, tears have a protective function of the cornea. Small organisms are washed out by tears, and some proteins in tears have anti-microorganism activity.

Since Josephson and Lockwood detected albumin, transferrin, ceruloplasmin, IgG and IgA in tears, extensive studies have been done of protein components in tears. Masson identified lactoferrin and lysozyme, and Sapse et al. confirmed the presence of ceruloplasmin, IgA, IgG, lysozyme and prealbumin. IgM and IgE were isolated from tears by Little et al. and Brauninger et al. All classes of immunoglobulin except IgD were identified in tears, so far.

IgA is a major immunoglobulin in tears. This IgA is a secretory IgA which is totally different from serum IgA, chemically and immunologically. IgA in tears decreased the adherence of the bacteria to the epithelium. IgA also plays a protective role against other infections. Isolation of specific IgA in tears against influenza virus and herpes virus suggests the antiviral function of IgA. IgA is also thought to be related to the development and severity of certain diseases, such as trachoma, ataxia telangiectasia, leprosy and ocular pemphigoid because the changes in the IgA level are related to the severity of these diseases.

IgA concentrations in normal human tears ranges from 14 to 50 mg/dl according to reports. This wide range presumably results from a difficulty in collecting tears and the low sensitivity of the measuring methods. The microcapillary method for collecting tears is time-consuming and needs an induction of tears by tear gas or bright light. Furthermore, the microcapillary tube is not easily available. Radial immunodiffusion (RIA) has been used to measure the IgA level in tears. However, this method has a lower sensitivity than the enzyme-linked immunosorbent assay (ELISA), and measurement of RIA is less reliable than that of ELISA. The author used strong absorbent, Weck

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Cel.\(^7\)\(^{18}\) to collect tears and used sandwich ELISA\(^{10,26}\) to measure the IgA concentration in tears. The results are presented.

**SUBJECTS AND METHODS**

*Subjects*

This study included 165 normal subjects (85 males, 80 females) who did not have any ocular and systemic diseases or any positive history of ocular surgery or chronic ocular medication. The ages ranged from 12 to 68 (average 38). Table 1 shows the demographic features of the study subjects.

<table>
<thead>
<tr>
<th>Table 1. Age and sex distribution</th>
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<tr>
<td>Age</td>
</tr>
<tr>
<td>10-19</td>
</tr>
<tr>
<td>20-29</td>
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<tr>
<td>30-39</td>
</tr>
<tr>
<td>40-49</td>
</tr>
<tr>
<td>50-59</td>
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<tr>
<td>60-69</td>
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<tr>
<td>Total</td>
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</table>

*Collection of tears*

Sterile Weck Cel\(^1\) (1 mm × 1 mm × 4 mm) was inserted into the inferior cul de sac of the subject without anesthesia. Attention was payed not to touch the cornea upon insertion, and the subject was instructed not to move his eyes in order to decrease reflex tearing and to protect the cornea. After the full saturation of Weck Cel\(^1\) with tears, the Weck Cel\(^1\) was removed from the cul de sac and transferred to one well of an ELISA plate (polystyrene plate, Costar\(^\dagger\)). A necessary amount of tears was aspirated with a micropipette when a fully saturated Weck Cel\(^1\) was squeezed in the well. When the ELISA was not performed immediately, the saturated Weck Cel\(^1\) was kept in a frozen state at −20°C.

*Enzyme-linked immunosorbent assay (ELISA)*

The sandwich ELISA method was used (Figure 1), and the brief method is described as follows. Each well of the polystyrene ELISA plate was coated with sheep anti-human secretory IgA (Cappel Laboratories Inc., alpha chain and secretory component specific) at a concentration of 10 \(\mu g/ml\). After that, a possible active area in the ELISA plate was blocked with a mixture of 3% bovine serum albumin, 0.05% Tween 20 and pH 7.2, 0.01M phosphate buffered saline. The tears of each subject were diluted in three different concentrations−1:100, 1:1,000 and 1:10,000. Fifty ml of each dilution was applied to the wells of the ELISA plate which already had a sheep anti-human secretory IgA coating, and this ELISA plate was incubated for one hour in 37°C. After that, peroxidase conjugated sheep anti-human secretory IgA (1:1,000 dilute, Cappel Laboratories Inc., alpha chain and secretory component specific) was applied. Phenylendiamine (0.04%) was added to produce a color reaction. A color spectrometer (Flow Lab.) was used to measure the color reaction. The mean optical density was obtained by averaging the two results of the same tear sample. An IgA concentration was calculated by using the Standard curve (Figure 2).
**Statistical analysis**

Student \( t \) test was used for statistical analysis.

**RESULTS**

The concentration of tear IgA in normal subjects was 60.5 ± 23.4 mg/dl. There was no significant difference in the IgA level between men (61.8 ± 23.2 mg/dl) and women (58.4 ± 23.5 mg/dl) (\( p > 0.05 \), Table 2).

**Table 2. Normal tear IgA level**

<table>
<thead>
<tr>
<th>Sex</th>
<th>IgA (mg/dl)</th>
<th>±S.D.*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>61.8</td>
<td>23.2</td>
</tr>
<tr>
<td>Female</td>
<td>58.4</td>
<td>23.5</td>
</tr>
<tr>
<td>Total</td>
<td>60.5</td>
<td>23.4</td>
</tr>
</tbody>
</table>

*: standard deviation

The concentrations of tear IgA in the teens, twenties, thirties, forties, fifties and sixties were 54.5 ± 24.4 mg/dl, 60.9 ± 21.3 mg/dl, 57.5 ± 21.0 mg/dl, 62.8 ± 22.7 mg/dl, 62.7 ± 24.5 mg/dl and 56.9 ± 23.6 mg/dl, respectively. There was also no significant difference among the different age groups (\( p > 0.05 \), Figure 3).

**DISCUSSION**

The ELISA is being used to measure small amounts of protein because of its high sensitivity and specificity.\(^{10,26}\) Immunoglobulins in tears are in very small quantity. The amount of IgA was reported to be 8.8-36.8 mg/dl,\(^{5-9,18,23,24}\) and the amount of IgM and IgE was less than 1 mg/dl.\(^{7,8}\)

These values are measured by radial immunodiffusion, which is a less accurate method in measuring these small amounts than the ELISA.

The first report using the ELISA to measure immunoglobulins in tears was published in 1983 by McGill.\(^{25}\) He used direct ELISA. However, the sandwich ELISA method was used to measure the tear IgA level in this study. Sandwich ELISA is more accurate than direct ELISA because in direct ELISA the incomplete attaching of IgA to the ELISA plate resulted in low value.\(^{10,26}\) This is the first measurement of tear IgA levels in normal Koreans by sandwich ELISA. The IgA concentration measured in this study was 60.5 mg/dl (mean), which is higher
than the value measured by radial immunodiffusion (8.8-36.8 mg/dl) and the value measured by direct ELISA (50-55 mg/dl).

There are several ways to collect tears, such as using a strong absorbent (Weck Cel+), microcapillary or Whatman paper. It may be impossible to collect tears without any irritation because there is only 7 µl of tears in the cul de sac. So, it is necessary to irrigate the eye for tear collection. But the best method of collecting tears is that of least irritation.

When Weck Cel+ was located in the deep portion of the inferior cul de sac, irritation was minimal as was the reflex tear production. In addition, large amounts (200-500 µl) of tears can be obtained by the Weck Cel+ method. The error in dilution of tears was minimized and repetitive tests were possible because of the large amount of collected tears.

It is still a matter of controversy whether or not reflex tears have more IgA than basal tears. Little and Bracciolini reported that reflex tears had more IgA. But McClellan et al. reported that there was no difference in the IgA level between reflex and basal tears. This needs further evaluation.

There was no statistically significant difference in the IgA level among several different age groups. This coincides with the results of Sen et al. and Kaufman, but not with the report of McGill et al., in which the level of IgA in tears decreased with age. It is not always true that the amount of immunoglobulins decreases with age, because the total amount of serum IgG and IgA increases even though their functions decrease with age.

Sandwich ELISA to measure the tear IgA level was established in this study, and further measurement of the tear IgA in different diseased states will be the next step.

REFERENCES