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ORIGINAL ARTICLE

Immunoglobulin Concentrations in Plasma and Saliva During the Neonatal Period

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Background: Screening for infectious diseases in newborns using immunoglobulin (Ig)A-, IgM-, and IgE-specific antibodies is expensive and impractical. To determine if total levels of these Igs can be used for screening purposes, thus simplifying the process, their basic levels in the 1st month of extrauterine life need to be determined. Additionally, the ability to simplify screening by using saliva also needs to be determined. The aim of this study was to determine IgA, IgM, and IgE concentrations in plasma and saliva in newborns, correlation between the samples, and relationship between Ig levels and newborn age.

Methods: We enrolled 53 apparently healthy newborns, paired samples of plasma and saliva were collected, and total IgA, IgM, and IgE concentrations determined by capture enzyme-linked immunosorbent assay. The correlation between plasma and saliva values was calculated by Spearman's rank correlation coefficient and the IgA, IgM, and IgE distributions were analyzed by the Shapiro-Wilk test. We also determined the level of each Ig concentration according to age.

Results: IgA and IgM levels in plasma and IgA levels in saliva increased significantly during 1st month of life, especially in the 2nd week and 3rd week, with a good correlation of IgA between plasma and saliva. IgE levels in both plasma and saliva and IgM levels in saliva were very low or absent.

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Conclusion: These results suggest that Igs in saliva could be good biomarkers for newborn screening programs during the 1st week of life. This study established reference values for Igs according to age in the neonatal period.

1. Introduction

Immunoglobulins (Igs) are humoral immunity molecules produced in response to internal and external antigens.[1] These proteins are synthesized *in utero* from the 1st week of gestation in response to maternal or other substances that cross the placenta, or as result of an active infection of the fetus.[2] Different Ig types are present in neonatal blood, with IgG being a mixture of antibodies synthesized by the fetus and those of the mother transferred by the chorionic villi FcRn receptor.[3] Conversely, maternal IgA, IgM, and IgE antibodies do not cross the placenta, therefore, these concentrations reflect the fetal immune response.[4]

Abnormal IgM, IgA, or IgE levels have been found as a result of fetal or newborn response to congenital infections, sepsis, allergies, stress, or exposure to environmental toxins (i.e., lead). Therefore, the detection of abnormal levels of these Igs could be useful for identification of health risks in the neonate.[5–9] In fact, postnatal screening programs for congenital infectious diseases, such as toxoplasmosis, have been implemented based on the search for specific antibodies associated with these Igs as primary biomarkers.[9,10]

Immunoglobulins are usually measured in venous or capillary blood, however, alternative samples, including saliva, have also been used for young children, given the non-invasive nature and ease of collection.[11] Specific antibodies against *Trypanosoma cruzi*, *Toxoplasma gondii*, and hepatitis B and C have been found in adult saliva, however, studies in newborns or infants using this sample as a biological matrix are scant.[12–15] Although total IgM and IgA levels have been measured in serum from the umbilical cord and 1-month-old infants, there are few data concerning their concentrations within the neonatal period.[16] Moreover, IgM and IgE levels in newborn saliva are practically unknown.[17–23] Therefore, the objective of this study was to determine total IgA, IgM, and IgE concentrations in the plasma and saliva of newborns, the correlation between samples, and the relationships between these levels and age.

2. Methods

2.1. Studied population and data collection

The study included a subset of newborns who participated in the large multicenter project, "Prenatal and neonatal screening for congenital infections in Mexico City". The study protocol was approved by the Research and Ethics Reviewing Board of Instituto Nacional de Pediatría, México, D.F., Mexico (registry number 002/2008). All procedures were explained to the parents or guardians, who signed an informed consent form. Apparently healthy children born at the Hospital General Dr. Manuel Gea González in Mexico City, Mexico were enrolled in this study. Only those cases with effective paired blood and saliva samples were included for the analysis. The exclusion criteria were the presence of maternal chronic disease, as well as a low Apgar score at birth ($<$ 6 at 1 minute or 5 minutes), a congenital abnormality, suspected infection, or transfusion with blood derivatives between birth and the day of sample collection.

Information on gestational age, health of mother and of child, and type of newborn feeding was obtained by an interview with the guardian and clinical data from the hospital.

2.2. Sample collection

All samples were collected from newborns following a 2 hour fast. Whole unstimulated saliva (100 μL) was obtained using sterile polypropylene transfer pipettes and placed in tubes with 3×10^{-4} g protease-inhibitor cocktail (Sigma P2714; Sigma-Aldrich, St. Louis, MO, USA). Samples were transported on ice to the laboratory where they were centrifuged at 176 g for 5 minutes and the supernatants stored at −80°C until analysis.

Blood samples were obtained by venopuncture using Becton Dickinson devices (Sigma-Aldrich). Blood was initially sampled in a heparinized Vacutainer tube (BD Bioscience, Franklin Lakes, NJ, USA), plasma separated by centrifugation, and aliquots prepared, frozen, and stored at −80°C until use.

2.3. Immunoglobulin quantification

Total IgA, IgM, and IgE concentrations in saliva and plasma were determined by capture enzyme-linked immunosorbent assay (ELISA). Briefly, 96-well plates (Maxisorb Nalge Nunc International, Rochester, NY, USA) were coated with 100 μL of the capture antibodies (anti-IgA, 2 μg/mL; anti-IgM, 0.125 μg/mL; and anti-IgE, 3 μg/mL) in 0.01M borate buffer (pH 8.0) and incubated at 4°C overnight. At each reaction step, three 5-minute washes with 200 μL 0.01M phosphate-buffered saline (PBS; pH 7.2) with 0.5% Tween 20 (PBS-T) and two 5-minute washes with PBS only were undertaken. The nonspecific-binding free sites were blocked with 1% albumin diluted in PBS-T (200 μL/well) by incubation at 37°C for 30 minutes.

Samples were processed by two-fold serial dilutions starting at ratios of 1:160, 1:500, and 1:40 for IgA, IgM, and