PLACENTAL TRANSFER OF TOTAL IGG AND IGG SUBCLASSES IN A TURKISH POPULATION LIVING IN EASTERN ANATOLIA

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ABSTRACT

Objective: *The aim of this comparative clinical study was to investigate the mother-to-baby transfer of immunoglobulin (Ig) G antibody in a Turkish population living in Eastern Anatolia.*

Material and Method: *One hundred three women, at various gestational ages ranging from 32 to 42 weeks, were enrolled to this mother-baby pair cross-sectional study. Levels of total IgG and the four subclasses of IgG were measured in serum samples taken after delivery by use of method of turbidimetric assay and radial immunodiffusion, respectively.*

Results: *When both term and preterm babies were considered cord serum had significantly higher total IgG and IgG1, while having significantly lower IgG2 and IgG3 levels than in maternal sera. The IgG1 were higher and IgG2 were lower in cord sera than in maternal sera for both full-term (37-42 weeks) and pre-term (32-36 weeks) matched pairs. The mean cord/maternal concentration ratio for IgG subclasses were decreased in the order of IgG1>IgG4>IgG3>IgG2 and IgG4>IgG1>IgG3>IgG2 for the full-term and pre-term matched pairs, respectively. No significant correlation was detected between total or subclasses IgG concentrations and gestational age.*

Conclusion: *In this studied Turkish population all four maternal IgG subclasses, IgG1 and IgG4 being more were transported across the placenta from mother to her fetus. The results are relevant to both vaccination and disease surveillance in early infancy.*

Key Words: *Fetal blood, immunoglobulin G, maternally-acquired immunity, immunity* ***Nobel Med 2012; 8(2): 59-64***

DOĞU ANADOLU'DA YAŞAYAN BİR TÜRK POPÜLASYONU ÖRNEĞİNDE TOTAL IgG VE IgG ALT TİPLERİNİN PLASENTAL TRANSFERİ

ÖZET

Amaç: Bu karşılaştırmalı klinik çalışmanın amacı Doğu Anadolu'da yaşayan Türk popülasyonunda anneden bebeğe immünglobülin (Ig) G antikor transfer düzeyini incelemektir.

Materyal ve Metod: Anne-bebek çiftinin kullanıldığı bu kesitsel çalışmaya gestasyonel yaşı 32-42 hafta arasında değişen toplam 103 kadın dahil edildi. Doğumu takiben alınan kan örneklerinde total IgG ve dört alt sınıfı turbidometrik analiz ve immünodiffüzyon yöntemleri ile ölçüldü.

Bulgular: Miyadında ve preterm bebekler bir arada tutularak yapılan değerlendirmede kord serumunda total IgG ve IgG1 düzeyi yüksek, IgG2 ve IgG3 düzeyleri ise maternal serumdan anlamlı düzeyde daha düşük bulundu. Miyadında (37-42 hafta) ve pretermlerde (32-36 hafta) kord serumunda maternal seruma göre IgG1 düzeyi yüksek IgG2 düzeyi ise düşüktü. Hem miyadında hem de pretermler için IgG alt sınıfları için ortalama kord/maternal konsantrasyon oranları sırasıyla IgG1>IgG4>IgG3>IgG2 ve IgG4>IgG1>IgG3>IgG2 şeklinde tespit edildi.

Total IgG veya IgG alt sınıfları ile gestasyonal yaş arasında anlamlı bir korelasyon saptanmadı (p>0,05).

Sonuç: Çalışılan Türk popülasyonunda IgG'nin 4 alt tipi de, IgG1 ve IgG4 daha etkin olmak üzere, plasenta yolu ile anneden bebeğe geçtiği tespit edilmiştir. Bu çalışmanın bulguları yenidoğanların bağışıklanması ve hastalık sürveyansı konusunda önem arz etmektedir.

Anahtar Kelimeler: Fetal kan, immunoglobulin G, anneden edinilmiş bağışıklık, bağışıklık **Nobel Med 2012; 8(2): 59-64**

INTRODUCTION

A newborn have a poorly developed immune system and is often unable to mount an effective immune response.[1] Newborns are generally protected by the antibodies they receive from their mother through the placenta before birth and through breast milk after birth. This passive immunity is necessary for them to meet the tremendous number of environmental challenges they will encounter in the early hours and days of life and helps them to fight off infections during the first several months of life until their own immune system is fully working. [1-4]

Immunoglobulins (Ig), or antibodies, are glycoprotein molecules produced by lymphocytes located in the bone marrow, a class known as B-lymphocyte.[3] Main functions of these multifunctional tools involve enhancing humoral immunity against bacterial, viral and other pathogens (therapeutic roles) and prevention of superimposed infections (prophylactic roles). IgG or gamma globulins (with 4 subclasses), carry long-term immunity.[5]

The placenta is the interface between a pregnant woman and the fetus she carries, presenting a selective barrier to the passage of various substances from the maternal to the fetal circulation and the passage of excretory products in the reverse direction. To enable this, placenta has wide range of transport processes including passive diffusion, active transport, pinocytosis and phagocytosis. There is also selective transfer of material from mother to fetus. These include maternal proteins such as immunoglobulin G, insulin and transcobalamin-vitamin B12 complex that are required for fetal development. [5-7]

The transfer of maternal IgG molecules to the fetus or infant is a mechanism by which mammalian neonates acquire humoral immunity to antigens encountered by the mother. The protein responsible for the transfer of IgG is the MHC class I-related receptor FcRn.[8] The IgG antibodies acquired from the mother provides passive immunity during the first few months of life. It has been documented that IgG class immunoglobulins are transported from the maternal blood to the fetus beginning as early as the 12th week of gestation. IgG concentrations in the cord blood remains low until between the 22nd to 26th weeks of gestation, but its concentrations in the cord blood exceeds those in the maternal sera at term.[8] IgG concentrations in the cord blood has a direct relationship to gestational age; hence prematurely born infants have lower serum IgG levels than term infants. At the time of birth, the IgG concentration in fetal blood often exceeds the maternal concentration, but this maternal originated IgG is gradually declines exponentially in the neonate, with very little remaining after the 4-6 months of life, when it starts to rise again owing to antibody synthesis by the infant.[3,9,10]

It is suggested that protection afforded by maternal-fetal transfer of IgG antibodies may vary between populations and under different environmental conditions. Different environments present different antigens to the individual and thus it is expected that→

populations exposed to different environments will have different immunoglobulin levels. Due to constant exposure to parasites and infectious agents, high total IgG levels are commonly determined in developing countries and this may underline the reported rapid decline in maternal antibodies in young infants in developing countries.[2,11,12]

Although all four subclasses of IgG are known to cross the placenta there are conflicting reports regarding the relative efficiency of transport of different IgG subclasses.[13,14] Determination of level of transfer of individual IgG subclasses is of importance since unrecognized immunodeficiency due to less efficiently transfer of some IgG subtypes might leave the newborn with increased susceptibility to certain infections.[15] Immunoglobulin levels are influenced by race, age and environment.[16]

Hence, this study was designed to investigate the transplacental transfer of IgG subclasses in relation to the gestational age in a population of term Turkish neonates.

MATERIAL and METHOD

Study Design: Informed consents were obtained from mothers for herself and for enrolment of the infant to assess the immunoglobulin levels, and the study design received ethical approval from the Local Ethics Committee. With informed Consent Form the patient was informed about the study.

This prospective research was performed in Fırat University Fırat Medical Centre. The institution serves a similar mixed urban and rural population in an eastern Anatolian region.

To assess the transplacental transfer of IgG subclass antibodies, mothers and their infants were recruited prospectively at our hospital. Parturients (≥32 weeks of gestation) who consented to participate were consecutively recruited at delivery in the labour ward. A total number of 103 mothers and their infants were studied. The inclusion criteria were gestational age between 32 to 42 weeks (assigned using maternal menstrual dates and confirmed by neonatal clinical assessment), no evidence of maternal infection during pregnancy and delivery, singleton birth and no laboratory evidence of infection. Essential and pregnancy-induced hypertension and diabetes were also among to the exclusion criteria.

All the mothers were healthy and all their babies had birth weights appropriate for gestational age. Pairs of mother and offspring with signs of infection, multiple pregnancy or malformations and mothers who had received blood transfusion in the 24 hours before delivery were excluded.

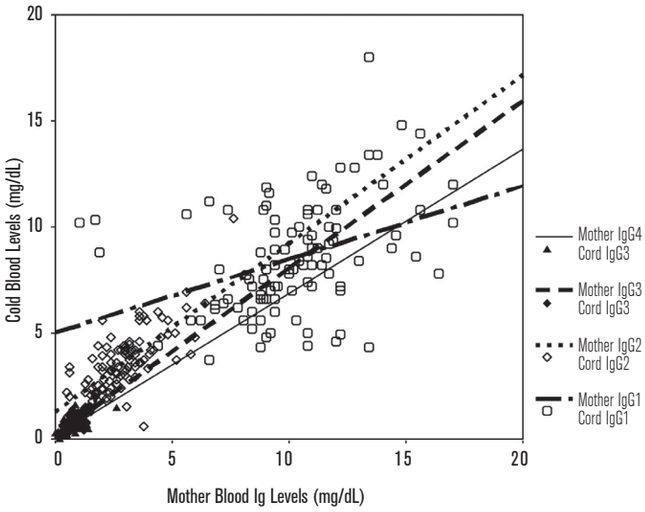

Figure 1. Scatter plot of maternal immunoglobulin concentrations against cord blood concentrations (r= 0.09, p=0.4).

To investigate the influence of gestational age on maternal-fetal and neonatal Ig level the subjects was stratified into 2 gestational age categories, 32 to 36 weeks (pre-term, n=16) and 37 to 42 weeks (full-term, n=87).

Serum collection: Blood samples were collected from 103 matched pairs of mothers and neonates within 48 hours after delivery. Peripheral venous blood from mothers and fetal blood from the clamped umbilical cord was collected. Sera separated from these samples were stored at -80°C until assayed.

Measurements: The levels of total IgG in serum were measured using an immuno-rate-turbidimetric method (immunoturbidometric method, Shiapparelli Biosystems, The Netherlands). The subclasses of IgG were estimated by single radial immunodiffusion using CLB PeliRIDe human IgG subclass RID kit (Amsterdam, The Netherlands). The IgG subclass concentrations in the test samples were determined according to protocol of the manufacturer. Total IgG and subclasses results were indicated as g/L.

Statistical Analysis: Data are presented as mean±SD. Tabulation and statistical analysis were carried out with SPSS (version 10.0) for Windows. Determining whether the data is normally distributed with Kolmogorow-Smirnov test was used. It was determined that the data does not conform to normal distribution. Mann-Whitney U test between groups, Wilcoxon Signed Ranks test for related samples. Spearman's test was used for examining the correlation between→

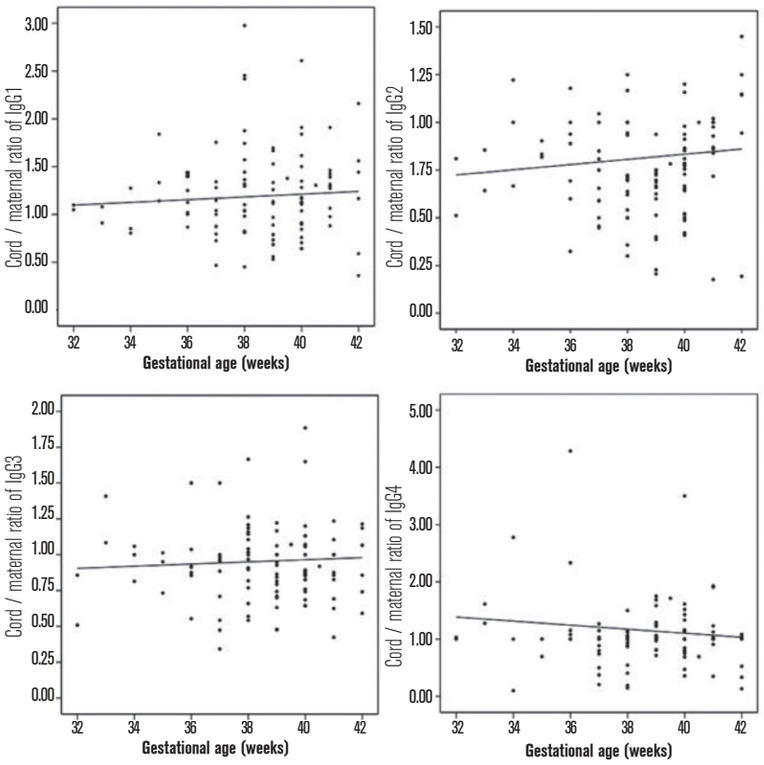

Figure 2. Correlation between cord/maternal ratio of subclasses of IgG based on gestational age. Upper left panel shows correlation between cord maternal ratio of IgG1 and gestational age (r= 0.09, p=0.4); upper right panel shows correlation between cord maternal ratio of IgG2 and gestational age (r= 1.2, p=0.2); lower left panel shows correlation between cord maternal ratio of IgG3 and gestational age (r=-0.01, p=0.9); lower right panel shows correlation between cord maternal ratio of IgG4 and gestational age (r= 0.02, p=0.8).

placental transfer of IgG subclasses and gestational age. p<0.05 was considered statistically significant.

RESULTS

The age of mothers varied between 17 and 46 years, with a mean age of 25.93±5.14 years. Gestational age ranged from 32 to 42 weeks. One hundred three mother-baby pairs were enrolled. The number of matched pairs of preterm (32-36 weeks) and full-term (37-42 weeks) pregnancies were 16 and 87, respectively.

The results of total IgG and subclasses of IgG for total study population are shown in Table 1.

The mean serum levels of total IgG were higher in cord sera than in maternal sera for both full-term (37-42 weeks) and pre-term (32-36 weeks) matched pairs, but these differences were not significant for pre-term group (Tables 1-2).

Gestational age stratification of the maternal-fetal-neonatal immunological parameters showed that; between 32-36 weeks gestation, the mean IgG2 antibody levels were significantly lower in cord sera than in maternal sera (p<0.01). Cord total IgG, IgG1, IgG3 and IgG4 antibody levels were not significantly different from their respective maternal levels (p>0.5, Table 2). Between 37-42 weeks gestation, the mean IgG1 and total IgG antibody levels were significantly higher in cord sera than in maternal sera (p<0.05 for IgG1, p< 0.01 for total IgG, Table 2); while IgG2 and IgG3 antibody levels were significantly lower in cord sera than in maternal sera (p<0.001 for IgG2, p<0.002 for IgG3, Table 2). Cord IgG4 antibody levels were not significantly different from the maternal levels (p>0.5, Table 2).

The mean IgG subclasses cord/maternal concentration ratios in pairs of maternal and cord sera at 32-36 weeks gestation and 37-42 weeks gestation were not significantly different (Table 3, Figure 1).

The mean cord/maternal concentration ratio for IgG subclasses were decreased in the order of IgG1>IgG4>IgG3>IgG2 and IgG4>IgG1>IgG3>IgG2 for the full-term and pre-term matched pairs, respectively (Table 3, Figure 1). There was no significant difference between pre-term and full-term matched pairs' with respect to the cord/maternal ratios of total IgG and IgG subclasses (p>0.05, Table 3). No significant correlation was detected between total or subclasses IgG concentrations and gestational age (Figure 2).

DISCUSSION

Transplacentally acquired maternal antibodies are fundamental for the immune defence of the neonate against infectious diseases during the period of immunologic immaturity.

In the present study we present results of measurements of the concentrations of endogenous antibodies including total IgG and subclasses of IgG in maternal and fetal sera at different gestational ages. This study demonstrates that transplacental transport varies with the subclass of IgG in Turkish population, IgG1 being the most, IgG2 being the least efficiently transported. These results also confirm that all four maternal IgG subclasses were transported across the placenta from mother to her fetus.

To our knowledge, this is the first study investigating placental transfer of IgG subclasses in a large population of matched pairs of Turkish mothers and neonates. A previous study was performed in Turkey but involving only 20 pairs of mother-cord blood.[17] They have reported IgG subclass values in Turkish population: IgG1 as 6.62 g/L, IgG2 as 3.77 g/L, IgG3 as 0.70 g/L, and no values were available for IgG4, which are similar to the values we determined except IgG1 being higher in the present study. →

The level of materno-fetal transmission of IgG and its four subclasses have been extensively investigated in different populations and have been found to be variable. Particular attention has been paid to IgG1 and IgG2; most of these studies have demonstrated higher levels of IgG1 and lower levels of IgG2 in cord sera than in maternal sera while levels of IgG3 and IgG4 being more variable. [8,18,19] In accord with other publications, we found that among to the IgG subclasses IgG2 was the least efficiently transferred from maternal to the neonatal blood in both term and preterm babies, as its levels in pre-term and full-term of neonates were significantly lower than their mothers.[8,10,18,20,21] Serum levels of IgG1 and total IgG were higher in neonatal blood than their maternal levels in both gestational periods between 32-36 weeks and 37-42 weeks and when all the data were pooled.

Possible correlation between the levels of IgG subclasses and gestational age has also been investigated. In a study performed on Japanese population by Hashira et al. it was found that the mean IgG1, IgG3 and IgG4 concentrations in cord sera were all significantly higher than in maternal sera at full-term gestation.[21] Lostal Gracia et al. reported that the relation between the average levels of maternal and cord serum was 1, 1.5, 1.9, and 0.48 for IgG1, IgG2, IgG3, and IgG4, respectively.[22] Similar to other publications, in our study the mean IgG1 concentrations in cord sera were significantly higher than in maternal sera at full-term gestation, but the IgG2 and IgG3, IgG4 levels were significantly lower in cord sera. In contrast to the previous reports, there was no significant correlation between gestational age and age and cord blood total IgG and IgG subclass levels. This is may be due to the lowest gestation included was 32 weeks and the level of transmission was sufficient by than. Indeed it has been demonstrated that by 26-34 weeks the intrauterine concentration of maternal IgG begin to rapidly increase due to maternal IgG transfer to the fetus. [23]

Conflicting results regarding the relative transport of the different IgG subclasses in full-term and pre-term gestations have been reported for different populations. While studies on a group of Gambian and Swiss mothers and newborns demonstrated lower levels of IgG1 and IgG2 subclasses lower in preterm infants than term infants, similar to our findings in a study on Brazilian mothers and newborns there was no significant difference between the pre-term and full-term gestations.[14,24,25] In this study we found the cord/maternal concentration ratios of IgG subclasses in full-term gestation as lgG1>IgG4>IgG3>IgG2. Garty et al. indicated that the efficiency of transplacental transfer of IgG1 and IgG4 was found to be significantly more efficient than that of IgG3 and IgG2.[13] Costa-Carvalho et al., Schur et al., Black et al. and Malek et al., Hashira et al. found this hierarchy as IgG1>IgG3>IgG4>IgG2.[10,21,25-27] Other hierarchies such as IgG1=IgG3=IgG4>IgG2, IgG1=IgG2=IG3>IgG4, IgG1=IgG4>IgG2=IgG3 and IgG1>IgG4>IgG2=IgG3 were demonstrated in several studies.[13,21] Our results are similar with study done by Garty et al. but different from other previous studies.[13] However, there are no big differences in this manner between the studies.

Although isolated finding of low concentrations of one or more IgG subclass does not identify a direct →

Table 1: Serum levels of total IgG and IgG subclasses in 103 pairs of maternal and cord sera at 32-42 weeks gestation (Mean±SD).

Total IgG and subclasses (g/L)	Maternal blood	Cord blood	p
IgG1	8.50±2.65	9.19±1.80	0.015
IgG2	3.65±1.45	2.71±1.18	0.001
IgG3	0.79±0.33	0.71±0.29	0.001
IgG4	0.45±0.37	0.40±0.26	0.95
Total IgG	11.55±4.23	12.58±4.87	0.004

Table 2: Serum levels of total IgG and IgG subclasses in pairs of maternal and cord sera according to the gestation weeks (Mean±SD).

Total IgG and subclasses (g/L)		Gestation weeks	Maternal blood	Cord blood	p
IgG subclasses	IgG1	32-36	8.60±2.05	9.33±1.26	0.2
		37-42	8.49±2.76	9.16±1.89	0.04
	IgG2	32-36	3.39±1.45	2.65±1.22	0.01
		37-42	3.69±1.45	2.72±1.18	0.0001
	IgG3	32-36	0.80±0.28	0.71±0.21	0.14
		37-42	0.79±0.34	0.71±0.30	0.001
	IgG4	32-36	0.47±0.37	0.44±0.27	0.18
		37-42	0.47±0.38	0.40±0.26	0.56
Total IgG		32-36	11.53±4.23	11.54±4.33	0.8
		37-42	11.53±4.23	12.77±4.96	0.003

Table 3: Cord/maternal IgG ratios according to the gestation weeks

Cord/Maternal IgG Ratios		Gestation weeks	n	Mean± SD
IgG subclasses	IgG1	32-36	16	1.27±0.26 NS
		37-42	87	1.20±0.49 NS
	IgG2	32-36	16	0.81±0.24 NS
		37-42	87	0.81±0.65 NS
	IgG3	32-36	16	0.95±0.26 NS
		37-42	87	0.95±0.40 NS
	IgG4	32-36	16	1.40±0.99 NS
		37-42	87	1.11±1.06 NS
Total IgG		32-36	16	1.00±0.32 NS
		37-42	87	1.20±0.59 NS

NS: Non significant

cause-and-effect relationship of increased susceptibility to infection, it has been shown that low IgG1 concentration is associated with primary or secondary immunodeficiency states. Low IgG2 concentration is associated with an increased risk of bacterial infections whereas isolated IgG3 and IgG4 deficiencies have not been convincingly demonstrated. [28,29] Furthermore, IgG1 and IgG3 are known to activate the complement system efficiently whereas IgG2 and IgG4 are poor complement activators and even IgG4 may itself inhibit complement activation.[30,31] Thus, these are of importance with respect to the possible deficiencies IgG3 and IgG4 in these infants unlikely to cause immunodeficiency and increased susceptibility to infection.

We conclude that placental transfer of IgG antibodies are similarly efficient above 32 weeks of gestational age and in this part of Turkey babies born after 32 week have adequate high concentration of serum IgG for immunity.

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