Anti-SARS-CoV-2 IgA and IgG in Human Milk From Vaccinated Mothers After Holder Pasteurization

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Abstract

Breastfeeding rendered crucial for preterm infants during COVID-19 pandemic. However, the role of donated human milk (DHM) in the protection against SARS-CoV-2 infection when own mother's milk is not available or insufficient remains unclear.

Pasteurization of DHM contributes to the loss of some biological and nutritional properties of human milk. The presence of specific breast milk SARS-CoV-2 antibodies has been demonstrated in women after COVID-19 disease and after vaccination. We aimed to evaluate the impact of Holder pasteurization on vaccinated donor women's milk and to identify the impact on the concentrations of specific immunoglobulins (Ig) against SARS-CoV-2.

A prospective, observational, exploratory pilot study in lactating women (n=12) who received the complete course of mRNA-based vaccines (BioNTech/Pfizer) against SARS-CoV-2 infection was conducted. Levels of antibodies directed to structural receptor-binding domain of the SARS-CoV-2 spike protein were determined by ELISA before and after pasteurization.

IgA and IgG anti-SARS-CoV-2 immunoglobulin concentrations were significantly reduced after Holder pasteurization. Interestingly, there was a negative correlation between the initial amount of anti-SARS-CoV-2 antibodies and the percentage of their recovery after the pasteurization for both isotypes. Despite the partial loss of immunoglobulins still a high percentage of antibodies remained after the pasteurization, a mean of 70.53 (3.4)% of anti-SARS-CoV-2 IgA and 81.99 (21.89)% of IgG antibodies. Our study underscores the potential relevance of breast feeding or alternatively DHM to provide babies at risk with virus-specific SARS-CoV-2 antibodies thus protecting them against COVID-19.

Introduction

On the 12nd March 2020 WHO declared COVID-19, the disease caused by the SARS-COV-2 virus, as a pandemic with an estimated mortality rate of 2.60% (1). Currently, there are still many questions about the COVID-19 and its evolution, prognosis, treatment, and interactions with the immune system that have not been yet answered. In this pandemic context, the mother-infant pair is a sensitive and vulnerable population (2). Breastfeeding is of crucial importance for both maternal and infant health. WHO states that human milk is the gold standard for feeding of human beings until the age of 2 years or beyond (3). Moreover, during pandemics mothers with COVID disease were encouraged to stay with their infants and breastfeed them as long as their clinical status allowed for it (2). Breast milk (BM) contains biological components including immunoglobulins, growth factors, hormones, cytokines, microorganisms that contribute to infants health and development (4). However, mothers who deliver premature infants often have difficulty to initiate the breastfeeding successfully (5). In the absence of own mothers' milk (OMM), there are two options, either the use of donated and pasteurized human milk (DHM) provided by a milk bank, or milk formula for premature babies (FM). Currently, most neonatology services are opting for the

administration of DHM (6) since it has demonstrated benefit in the protection against necrotizing enterocolitis (7, 8) compared to FM.

Nevertheless, DHM is pasteurized in milk banks to eliminate potential pathogenic agents. This mandatory process causes the loss of some of the biological, structural and functional properties of BM (9). Of note, after maternal recovery from COVID-19 and after vaccination there is maternal-infant transmission of antibodies through BM (10, 11). However, it remains unclear whether these antibodies persist after Holder pasteurization (which involves heating DHM at 62.5°C for at least 30 minutes) and could contribute to the passive protection of offspring.

The aim of this work was to evaluate the impact of pasteurization on the concentrations of specific immunoglobulins (Ig) against SARS-CoV-2 in milk from women who were vaccinated against COVID-19 with mRNA-based vaccines.

Materials And Methods

Study Design and volunteers

This is a prospective, observational study in lactating women (n = 12) who received the complete course of vaccination against SARS-CoV-2 infection in Spain (Trial Registration: NCT04751734). All participants were recruited in the Autonomous Community of Valencia (Spain) from January to April 2021 and as health care workers pertained to the vaccination priority groups. Lactating women enrolled in the study received two doses of the mRNA vaccine BioNTech/Pfizer (BNT162b2 mRNA). Other clinical and demographic data were also collected from the participants (See Table 1). All volunteers received oral and written information about the study and written consent was obtained. All procedures were in accordance with the ethical standards approved by the Ethical Committee of the Hospital Clínico Universitario de Valencia (Ref. 2020/133) and the Consejo Superior de Investigaciones Científicas [CSIC for National Research Council of Spain (Ref. 061/2021)].

Table 1
Demographics and clinical characteristics of women vaccinated against SARS-CoV-2.

	BioNtech/Pfizer N = 12
Maternal characteristics	
Age (years) mean $\pm$ SD	35,2 $\pm$ 4,5
Chronic diseases (hypothyroidism, heart disease, arterial hypertension, obesity, mellitus diabetes, etc.) (%)	0 (0)
Medication (%)	0 (0)
SARS-CoV-2 positive PCR before vaccine (%)	0 (0)
Infant characteristics	
Gender (female, %)	8 (66,6)
Mode of birth (C-section, %)	2 (16,7)
Gestational age (weeks), mean $\pm$ SD	40 $\pm$ 0,74
Weight at birth (g), mean $\pm$ SD	3,46 $\pm$ 0,44
Length at birth (cm), mean $\pm$ SD	50,75 $\pm$ 1,4
Infant's age at maternal 1st vaccine dose (month), mean $\pm$ SD	11,2 $\pm$ 9,7
Weight at maternal 1st vaccine dose (kg), mean $\pm$ SD	8,5 $\pm$ 2,3
Infant age at maternal 2nd vaccine dose (month), mean $\pm$ SD	13,2 $\pm$ 7,1
Weight at maternal 2nd vaccine dose (kg), mean $\pm$ SD	9,3 $\pm$ 2,1
Exclusive breastfeeding at maternal vaccination (%)	5 (41,7)
Secondary effects of the vaccine in the neonates (%)	0 (0)

Breast milk collection and processing

BM was collected following a standardized protocol described elsewhere preferably in the morning (12). For the standardized milk collection, breast skin was cleaned with 0.5% chlorhexidine solution and first drops were discarded. Then, BM was collected using a sterile pump in sterile bottles to normalize the milk collection. Samples included in the analysis were collected 15–20 days after the second dose of SARS-CoV-2 vaccine when the maximum immunological response in terms of antibody production has been reported (13–15). Samples were kept at -20°C until transferred to the laboratory where they were stored at -80°C until further analysis.

Samples were divided in two aliquots (10 mL) and one of them was pasteurized in the regional milk bank of Valencia (16) using Holder protocol which consists in a heating process at 62.5°C during 30 min (17). Then, milk whey was obtained from both milk aliquots by centrifugation at 11000g at 4°C during 15 min.

Breast milk SARS-CoV-2-specific antibody detection

Levels of antibodies before and after pasteurization were analyzed. Antibodies directed to structural receptor-binding domain (RBD) of the SARS-CoV-2 spike protein were determined in milk by ELISA as previously described (13). Samples were diluted 1:4 before analysis and a standard curve was included in each plate to facilitate the analysis of the immunoglobulin levels. The standard curve consisted in ten 3-fold serial dilutions from a mixture of samples ($n = 10$) that had been tested in previous studies (13, 14), and showed higher levels of both Ig concentrations. These milk samples were collected from mothers vaccinated against SARS-CoV-2 at 20 days after the second dose since a previous study showed higher levels of antibodies in that period (14).

An arbitrary concentration unit (AU) of 3000 was assigned to the highest OD value corresponding to the undiluted sample.

Statistical Analysis

All statistical analysis were performed using GraphPad Prism (v. 8.4.3) (GraphPad Software, La Jolla CA, US). Antibody kinetics were fitted using a nonlinear 4-parameter least-square fit in this statistical software. The AU of anti-SARS-CoV 2 IgG and IgA in the tested milk samples were then interpolated from the standard curve using the calculated fit.

The levels of anti-SARS-CoV-2 IgG and IgA in BM samples were compared before (pre-P) and after (post-P) pasteurization. Paired Wilcoxon test was used to assess the significance in the difference of the AU in pre- and post-pasteurization samples. The same statistical test was performed for the comparison of the percentage of anti-SARS-CoV-2 IgG and IgA that remained in BM after pasteurization.

Results

Study population characteristics

The study included twelve lactating women receiving COVID-19 vaccination with a mean age of 35 years and their offspring at a mean age of 11 months. Maternal demographics and clinical characteristics of women vaccinated against SARS-CoV-2 ($n = 12$) are described in Table 1. The infants' mean and standard deviation weight when 1st dose was administered to the mothers was 8.5 ± 2.3 kg and 66,6% were females. None of the infants developed fever after maternal vaccination. There were no serious adverse events during the study period.

SARS-CoV-2 reactive antibodies in breast milk after pasteurization

Both isotypes of anti-SARS-CoV-2, IgA (Fig. 1A; $p = 0.009$) and IgG (Fig. 1B; $p = 0.007$), significantly decreased after Holder pasteurization. Interestingly, there was a negative correlation between the initial amount of anti-SARS-CoV-2 antibodies and the percentage of their recovery after the pasteurization in both isotypes (IgA, $\rho: -0.78$, $p = 0.049$; IgG, $\rho: -0.25$, $p = 0.430$) (Fig. 1C-D).

Despite the decrease in concentration of IgA and IgG still a high percentage of antibodies remained after the pasteurization process (Fig. 1E). A mean (standard deviation) of 70.53 (3.4)% of anti-SARS-CoV-2 IgA and 81.99 (21.89)% of IgG antibodies were detected after pasteurization. Indeed, the percentage of lost antibodies after the procedure seemed to be lower in terms of IgG compared to IgA, although this difference was not statistically significant ($p = 0.233$).

Discussion

Our study shows a significant reduction of Anti-SARS-CoV-2 IgA and IgG immunoglobulin in BM from vaccinated women after pasteurization. Nevertheless, a high percentage of these antibodies (between 70–80% of antibodies depending on IgA or IgG) remained in DHM after the pasteurization. Our results suggest that DHM could confer a potential protection against SARS-CoV-2 should OMM be unavailable. However, the functionality of the remaining immunoglobulins after pasteurization is yet unknown.

Breastfeeding and its potential impact on neonatal health is especially relevant in the context of prematurity one of the leading causes of neonatal mortality worldwide (18, 19). As mothers with preterm birth often have difficulties in breastfeeding (5), most neonatal services are opting for the administration of donor human milk (27) due to its proven benefit protecting against necrotizing enterocolitis (7, 20). While Holder pasteurization (62.5°C, 30 min) ensures the inactivation of non-heat-resistant viruses, including coronaviruses, if present (21), it also causes the loss of some of the biological, structural and functional properties of fresh milk (9, 17, 22) as it triggers cellular (including B and T lymphocytes) and bacterial destruction, as well as partial or total alteration of the structure and function of some of its components. Thus, to evaluate the effect of pasteurization on the potential protective capacity of breastmilk against viral infection will be especially relevant for its prevention in these especially vulnerable infants. Our results agree with Peila et al. (17), which showed the great impact of Holder pasteurization on the antibodies' levels. This effect has been also previously observed in total IgA, IgM, cytokines, growth factors and other components (17, 23, 24).

Upon maternal infection with SARS-CoV-2, a rapid and strong antibody response is induced with subsequent accumulation of substantial amounts of specific neutralizing secretory IgA (sIgA) in BM (25, 26). Other studies have also reported the presence of specific antibodies in milk (27, 28). Favara et al. confirmed strongly neutralizing IgA and IgG antibodies against multiple SARS-CoV-2 antigens in BM and serum at 2.5 and 6.5 months post-infection (27).

High levels of specific antibodies after immunization against other viruses, like influenza virus, have been shown (29). Indeed, first studies showed the existence of anti-SARS-CoV-2 antibodies in BM of women vaccinated with mRNA vaccines (11, 30). Furthermore, a study conducted by our research group showed

that HM from vaccinated women contained RBD-specific IgA and IgG, with levels increasing significantly after the second dose and decreasing after 3 weeks (14). Interestingly, IgG levels in vaccinated women were significantly higher than those observed in the milk of COVID-19 infected and/or recovered women. These results are in agreement with previous data showing levels of antibodies against SARS-CoV-2 in milk from vaccinated women (14). These results suggested the efficiency of vaccines to generate an IgG response (13). The presence of antibodies highlights the potential ability of BM to protect infants against coronavirus infection and underscores the essential role of breastfeeding also in the context of pandemics.

However, the neutralizing capacity of anti-SARS-CoV-2 antibodies in BM hasn't been sufficiently explored. In a systematic review, Low et al., concluded that fifty percent of mothers with COVID-19 harbored antibodies in milk capable of neutralizing SARS-CoV-2 infectivity *in vitro* (31). They suggested that pasteurization of BM would not decrease SARS-CoV-2 antibody titers but its neutralizing capacity (31).

One of the limitations of our study is the lack of determination of the SARS-CoV-2 neutralizing capacity of the antibodies detected after pasteurization. Other limitations could include the low sample size and the lack of information on virus-specific antibodies in blood from neonates. As proposed in the literature, breastfeeding offers protection and passive immunity to infants, but it remains to be investigated whether this effect persists after pasteurization. However, our study sheds light on the presence of antibodies in HDM after pasteurization and ensures future research in the field. There are still many open questions including when SARS-CoV-2 antibodies are produced after maternal vaccination, when they can be detected in BM and how long they persist. Indeed, the actual effectiveness of these antibodies in milk is not really known and it remains to be investigated whether the antibodies are well absorbed in the intestine of infants and pass into the bloodstream and exert a protective function.

Conclusions

The presence of anti-SARS-CoV-2 antibodies suggests that BM might have a protective effect in newborns. Despite a decrease in IgA and IgG concentrations in human milk after Holder pasteurization, a significant percentage of antibodies remains detectable. Our study endorses the relevance of breastfeeding during the pandemic and highlights the potential relevance of virus-specific SARS-CoV-2 antibodies providing passive immunity to breastfed infants, protecting them against COVID-19. Our study supports the official recommendations about the safety of breast feeding during the COVID-19 pandemic, indicating that breastfeeding should be a priority with potential benefit for both mothers and neonates. Further studies are needed to clarify the effectiveness of these antibodies in breastfed infants as well as how long they remain effective.

Declarations

Ethics approval and consent to participate:

All procedures were in accordance with the ethical standards approved by the Ethical Committee of the Hospital Clínico Universitario de Valencia (Ref. 2020/133) and the Consejo Superior de Investigaciones Científicas [CSIC for National Research Council of Spain (Ref. 061/2021)].

CONSENT FOR PUBLICATION:

All authors agree with the publication of the results obtained.

AVAILABILITY OF DATA AND MATERIALS:

all the data can be exposed for validation.

FUNDING AND CONFLICT OF INTEREST:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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AUTHOR'S CONTRIBUTIONS:

AP-L, MG, MS-R, planned the experiments. AP-L, MG, AR-B, MC and MV-E collected the samples and clinical data. AP-L, MG, MV, CMC and MCC requested the ethical approvals. MS-R and CB performed the ELISA analysis and analyzed data. AP-L and M-SR wrote first draft, and all authors reviewed the manuscript. All authors approved the final version the manuscript.

Conflicts of interest:

Authors declare not having conflicts of interest

References

1. WHO Coronavirus (COVID-19) Dashboard [Internet]. [cited 2021 Aug 26]. Available from: <https://covid19.who.int>

2. Bhatt H. Should COVID-19 Mother Breastfeed her Newborn Child? A Literature Review on the Safety of Breastfeeding for Pregnant Women with COVID-19. *Curr Nutr Rep.* 2021 Mar;10(1):71–5.
3. Saadeh MR. A new global strategy for infant and young child feeding. *Forum Nutr.* 2003;56:236–8.
4. Stuebe A. There Is No Formula for Human Milk. *Breastfeed Med Off J Acad Breastfeed Med.* 2020 Sep;15(9):602.
5. Wilson E, Edstedt Bonamy A-K, Bonet M, Toome L, Rodrigues C, Howell EA, et al. Room for improvement in breast milk feeding after very preterm birth in Europe: Results from the EPICE cohort. *Matern Child Nutr.* 2018;14(1).
6. ESPGHAN Committee on Nutrition, Arslanoglu S, Corpeleijn W, Moro G, Braegger C, Campoy C, et al. Donor human milk for preterm infants: current evidence and research directions. *J Pediatr Gastroenterol Nutr.* 2013 Oct;57(4):535–42.
7. Quigley M, Embleton ND, McGuire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. *Cochrane Database Syst Rev.* 2019 19;7:CD002971.
8. Brown JVE, Walsh V, McGuire W. Formula versus maternal breast milk for feeding preterm or low birth weight infants. *Cochrane Database Syst Rev.* 2019 12;8:CD002972.
9. Baro C, Giribaldi M, Arslanoglu S, Giuffrida MG, Dellavalle G, Conti A, et al. Effect of two pasteurization methods on the protein content of human milk. *Front Biosci Elite Ed.* 2011 Jun 1;3:818–29.
10. Valcarce V, Stafford LS, Neu J, Cacho N, Parker L, Mueller M, et al. Detection of SARS-CoV-2-Specific IgA in the Human Milk of COVID-19 Vaccinated Lactating Health Care Workers. *Breastfeed Med Off J Acad Breastfeed Med.* 2021 Aug 20;
11. Perl SH, Uzan-Yulzari A, Klainer H, Asiskovich L, Youngster M, Rinott E, et al. SARS-CoV-2-Specific Antibodies in Breast Milk After COVID-19 Vaccination of Breastfeeding Women. *JAMA.* 2021 May 18;325(19):2013–4.
12. García-Mantrana I, Alcántara C, Selma-Royo M, Boix-Amorós A, Dzidic M, Gimeno-Alcañiz J, et al. MAMI: a birth cohort focused on maternal-infant microbiota during early life. *BMC Pediatr.* 2019 May 3;19(1):140.
13. Bäuerl C, Randazzo W, Sánchez G, Selma-Royo M, Garcia-Verdevio E, Martínez L, et al. SARS-CoV-2 RNA and antibody detection in breast milk from a prospective multicentre study in Spain. *Arch Dis Child Fetal Neonatal Ed.* 2021 Aug 20;fetalneonatal-2021-322463.
14. Selma-Royo M, Bäuerl C, Mena-Tudela D, Aguilar-Camprubí L, Pérez-Cano FJ, Parra-Llorca A, et al. Anti-Sars-Cov-2 IgA And IgG In Human Milk After Vaccination Is Dependent On Vaccine Type And Previous Sars-Cov-2 Exposure: A Longitudinal Study. *medRxiv.* 2021 May 23;2021.05.20.21257512.
15. Chambers C, Krogstad P, Bertrand K, Contreras D, Tobin NH, Bode L, et al. Evaluation for SARS-CoV-2 in Breast Milk From 18 Infected Women. *JAMA.* 2020 Oct 6;324(13):1347.
16. Aceti A, Corvaglia L, Faldella G. Human milk banks: lights and shadows. *J Pediatr Neonatal Individ Med JPNIM.* 2014 Oct 3;3(2):e030225.

17. Peila C, Moro GE, Bertino E, Cavallarin L, Giribaldi M, Giuliani F, et al. The Effect of Holder Pasteurization on Nutrients and Biologically-Active Components in Donor Human Milk: A Review. *Nutrients*. 2016 Aug 2;8(8).
18. Yismaw AE, Gelagay AA, Sisay MM. Survival and predictors among preterm neonates admitted at University of Gondar comprehensive specialized hospital neonatal intensive care unit, Northwest Ethiopia. *Ital J Pediatr*. 2019 Jan 7;45(1):4.
19. Glass HC, Costarino AT, Stayer SA, Brett CM, Cladis F, Davis PJ. Outcomes for extremely premature infants. *Anesth Analg*. 2015 Jun;120(6):1337–51.
20. Boyd CA, Quigley MA, Brocklehurst P. Donor breast milk versus infant formula for preterm infants: systematic review and meta-analysis. *Arch Dis Child Fetal Neonatal Ed*. 2007 May;92(3):F169-175.
21. Unger S, Christie-Holmes N, Guvenc F, Budykowski P, Mubareka S, Gray-Owen SD, et al. Holder pasteurization of donated human milk is effective in inactivating SARS-CoV-2. *CMAJ Can Med Assoc J J Assoc Medicales Can*. 2020 Aug 4;192(31):E871–4.
22. Aceti A, Cavallarin L, Martini S, Giribaldi M, Vitali F, Ambretti S, et al. Effect of Alternative Pasteurization Techniques On Human Milk's Bioactive Proteins. *J Pediatr Gastroenterol Nutr*. 2019 Dec 24;
23. Escuder-Vieco D, Espinosa-Martos I, Rodríguez JM, Fernández L, Pallás-Alonso CR. Effect of HTST and Holder Pasteurization on the Concentration of Immunoglobulins, Growth Factors, and Hormones in Donor Human Milk. *Front Immunol*. 2018;9:2222.
24. Adhisivam B, Vishnu Bhat B, Rao K, Kingsley SM, Plakkal N, Palanivel C. Effect of Holder pasteurization on macronutrients and immunoglobulin profile of pooled donor human milk. *J Matern-Fetal Neonatal Med Off J Eur Assoc Perinat Med Fed Asia Ocean Perinat Soc Int Soc Perinat Obstet*. 2019 Sep;32(18):3016–9.
25. Pace RM, Williams JE, Järvinen KM, Belfort MB, Pace CDW, Lackey KA, et al. Characterization of SARS-CoV-2 RNA, Antibodies, and Neutralizing Capacity in Milk Produced by Women with COVID-19. *mBio [Internet]*. 2021 Feb 9 [cited 2021 Jun 7];12(1). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7885115/>
26. Fox A, Marino J, Amanat F, Oguntuyo K, Hahn-Holbrook J, Lee B, et al. The Spike-specific IgA in milk commonly-elicited after SARS-Cov-2 infection is concurrent with a robust secretory antibody response, exhibits neutralization potency strongly correlated with IgA binding, and is highly durable over time [Internet]. 2021 Mar [cited 2021 Sep 11] p. 2021.03.16.21253731. Available from: <https://www.medrxiv.org/content/10.1101/2021.03.16.21253731v1>
27. Favara DM, Ceron-Gutierrez ML, Carnell GW, Heeney JL, Corrie P, Doffinger R. Detection of breastmilk antibodies targeting SARS-CoV-2 nucleocapsid, spike and receptor-binding-domain antigens. *Emerg Microbes Infect*. 2020 Dec;9(1):2728–31.
28. Demers-Mathieu V, DaPra C, Mathijssen G, Sela DA, Järvinen KM, Seppo A, et al. Human Milk Antibodies against S1 and S2 Subunits from SARS-CoV-2, HCoV-OC43, and HCoV-229E in Mothers

with a Confirmed COVID-19 PCR, Viral SYMPTOMS, and Unexposed Mothers. *Int J Mol Sci.* 2021 Feb 9;22(4):1749.

29. Schlaudecker EP, Steinhoff MC, Omer SB, McNeal MM, Roy E, Arifeen SE, et al. IgA and neutralizing antibodies to influenza a virus in human milk: a randomized trial of antenatal influenza immunization. *PloS One.* 2013;8(8):e70867.
30. Gray KJ, Bordt EA, Atyeo C, Deriso E, Akinwunmi B, Young N, et al. Coronavirus disease 2019 vaccine response in pregnant and lactating women: a cohort study. *Am J Obstet Gynecol* [Internet]. 2021 Mar 25 [cited 2021 Jun 7];0(0). Available from: [https://www.ajog.org/article/S0002-9378\(21\)00187-3/abstract](https://www.ajog.org/article/S0002-9378(21)00187-3/abstract)
31. Low JM, Low YW, Zhong Y, Lee CYC, Chan M, Ng NBH, et al. Titres and neutralising capacity of SARS-CoV-2-specific antibodies in human milk: a systematic review. *Arch Dis Child Fetal Neonatal Ed.* 2021 Jul 13;fetalneonatal-2021-322156.

Figures

Figure 1

Variation of anti-SARS-CoV-2 antibody levels in breast milk after Holder pasteurization. Panels A-B. Comparison of immunoglobulin A (A) and G (B) antibody levels before (Pre-P) and after (Post-P) pasteurization. Panel C-D. Spearman's rank correlation between the initial levels of Ig in log-transformed arbitrary units (AU) and the percentage of remaining Ig respecting the initial. Panel E. Comparison

between the remaining immunoglobulin percentages after the pasteurization process according to immunoglobulin isotype. Wilcoxon matched pairs signed rank test was used to determine the significance of the difference between both isotypes.