NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

DETECTION OF SARS-COV-2 SPESIFIC IgA ANTIBODY IN THE HUMAN MILK OF COVID-19 VACCINATED LACTATING WOMEN

M.Sc. THESIS

Şuheda Nur İNECİ

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Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

ŞUHEDA NUR İNECİ

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ŞUHEDA NUR İNECİ

Abstract

Detection Of Sars-COV-2 Spesific Iga Antibody In The Human Milk Of Covid-19 Vaccinated Lactating Women

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In November 2019, COVID-19, caused by SARS-CoV-2, spread beyond the world, and women with vaccines developed to prevent it can pass the antibodies they have gained after the vaccine to their infants as passive immunity by breastfeeding. In this study, we aimed to see the transfer of SARS-CoV-2 specific IgA antibody to infants from breast milk collected from each of the mothers who were hospitalized in the Near East University Hospital, who received the COVID 19 vaccine. Of the 23 samples, 4 (17.4%) were vaccinated with CoronaVac and 19 (82.6%) with Pfizer/Biontech, and the breast milk sIgA value for Coronavac was 0.24±0.13. The sIgA value for the Pfizer/Biontech group was found to be 1.84±2.17, and the IgA value in breast milk increased after vaccination. We provided the Anti-SARS-CoV 2 ELISA IgA kit for our study. To use the ELISA method, 30 ml of breast milk was collected from 23 patients in the Near East University Hospital.

Key Words: IgA, SARS-CoV-2, passive immunity, vaccine, breast milk.

Özet

Covid-19 Aşısı Olan Emziren Kadınların İnsan Sütünde Sars-COV-2 Spesifik IgA Antikor Tespiti

İneci, Şuheda Nur

Yüksek Lisans, Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji Anabilim Dalı

Danışman: Prof. Dr. Hüseyin Kaya Süer

Şubat, 2023, 95 Sayfa

Kasım 2019'da, SARS-CoV-2'nin neden olduğu Covid-19 dünyanın ötesine yayıldı ve bunu önlemek için geliştirilen aşıları olan kadınlar, aşı sonrası kazandıkları antikorları emzirme yöntemiyle bebeklerine pasif bağışıklık olarak aktarabilirler.

Bu çalışmada, Kovid 19 aşısı olmuş, Yakın Doğu Üniversitesi Hastanesi'nde yatmakta olan annelerin herbirinden toplanan anne sütünden bebeklere SARS-CoV-2 spesifik IgA antikorunun aktarımının görülmesini amaçladık. 23 örnekten 4'ü (%17,4) CoronaVac, 19'u (%82,6) Pfizer/Biontech ile aşılanmış olup Coronavac için anne sütü sIgA değeri 0.24±0.13 olarak bulunmuştur. Pfizer/Biontech grubu için sIgA değeri 1.84±2.17 bulunmuş olup aşı sonrası anne sütünde IgA değeri artmıştır. Çalışmamız için Anti-SARS-CoV 2 ELISA IgA kitini temin ettik. ELISA yöntemi kullanılmak üzere, Yakın Doğu Üniversitesi Hastanesi'ndeki 23 hastadan 30 ml anne sütü toplandı.

Anahtar Kelimeler: IgA, SARS-CoV-2, pasif immünite, aşı, anne sütü.

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List of Abbreviations

°C:	Celsius
µl:	Microliter
mL:	Milliliter
nm:	Nanometer
g:	Centrifuge
Ml:	Milligrams
SARS CoV 2:	Acute Respiratory Syndrome Coronavirus 2
WHO:	World Health Organization
MERS:	Middle East Respiratory Disease
Ig:	Immunoglobin
SIgA:	Secretory Immunoglobin IgA
SARI:	Severe Acute Respiratory Infection
HCoV:	Human Coronavirus
S:	Spike
M:	Membrane
E:	Envelope
N:	Nucleocapsid
HMO:	Human Milk Oligosaccharides
HAMLET:	Human Lactalbumin Rendered Deadly To Tumor Cells
LF:	Lactoferrin
LPO:	Lactoperoxidase
Th1:	T Helper Type 1

CI: Confidence Interval

VE: Vaccination Efficacy

PAMPs: Pathogen Associated Molecular Pattern Molecules

TLR: Tool Like Receptor

MUC: Mucin

MHC: Major Histocompability Complex

IFN: Interferon

LPS: Lipopolysaccharide

TNF: Tumor Necrosis Factor

LT: Lymphotoxin

TGF: Transforming Growth Factor

FOXP3: Forkhead Box P3 Protein

BNT162b2: Pfizer Biontech Vaccine

VLA2001: Valvena Vaccine

ChAdOx1-S: Oxford-Astrazeneca Vaccine

NVX: Novavax Vaccine

CpG: Cytosine Phosphor Guanine

CHAPTER I

Introduction

In December 2019, Wuhan, China, saw a series of serious unusual respiratory illnesses that later spread to neighboring cities (Xiong et al., 2020). Soon later, experts reached the conclusion that the new coronavirus is to blame for the illness. This novel coronavirus resembled the SARS-CoV which was the reason to cause the SARS outbreak in between 2002 and 2003 (Peret et al., 2003). The condition brought on by this virus is known as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2/ 2019-nCoV/ COVID-19) by the World Health Organization (WHO). A beta coronavirus, such as this one, can cause illnesses like SARS and MERS (Chan et al., 2015; Elfiky et al., 2017; Shreen et al., 2020).

Since the beginning of the pandemic, supportive measures have been the backbone of therapy; more than one hundred vaccine candidates are now through various stages of clinical testing to prevent COVID-19. mRNA vaccines (Pfizer-BioNTech [Comirnaty], and Moderna), replication-defective adenoviral vector vaccines (Johnson & Johnson's Janssen, and AstraZeneca), sub-unit/protein-based vaccines (Novavax), DNA vaccines, and inactivated vaccines (Valneva, and Sinovac-CoronaVac) are some of the vaccine candidate types that are currently under research (Kaur & Gupta, 2020; Ura et al., 2020).

The term "immune system" refers to a group of cells, substances, and mechanisms that work to defend the skin, respiratory passages, digestive tract, and other organs against external antigens including viruses, cancerous cells, poisons, and microbes (organisms like bacteria, fungus, and parasites). The

immune system may be conceptualized into two lines of defense: innate and acquired immune system (Marshall et al., 2018). The humoral immune response is the basic component of acquired immunity against viral infections. Success of the vaccination is thought to be significantly influenced by the rate of production, size, and duration of the antibody-mediated humoral immune response that arises against the various viral proteins of SARS-CoV-2. The primary purpose of most vaccines is the stimulation of neutralizing antibodies due to their potential to reduce disease severity (Plotkin,2020).

One of the five principal immunoglobulins, immunoglobulin A (IgA), is essential for maintaining mucosal homeostasis in the gastrointestinal, respiratory, and genitourinary tracts. In this capacity, IgA serves as the dominant antibody of immunity (Breedveld & Van Egmond, 2019). It plays a critical part in the body's defense against antigens being the second most prevalent immunoglobulin type (Mkaddem et al., 2014).

Human milk has long been known to offer health advantages. It has been shown that human milk, as opposed to formula feeding, lowers morbidity and mortality, mostly because infections, including as respiratory infections, are less common (Lamberti et al., 2013). An infant's immune system is still developing throughout the first six months of life, which limits its capacity to mount a potent immunological response. Fortunately, the mother passes these antibodies to the child through human milk. The most common antibody found in human milk, secretory immunoglobulin A (IgA), is essential for mucosal immunity as a first line of defense against many illnesses (Schlaudecker et al., 2013).

This study aims to investigate the presence of SARS-CoV-2 specific IgA immunoglobins in human milk of breastfeeding women following vaccination. The ELISA method was used to obtain the results.

CHAPTER II

Literature Review

2.1. Coronavirus disease 2019 (COVID-19)

A worldwide outbreak of serious critical respiratory infection (SARI) occurred in the spring of 2003 (K. L. E. Hon et al., 2003; Sonja, 2020; K. L. Hon, 2009). The World Health Organization created the name SARS and classified the relevant coronavirus SARS-CoV. Winter 2019 had seen the commencement of a SARI outbreak in Wuhan, China, which quickly spread worldwide. Another new coronavirus was found to be the perpetrator; Due to illness's similarities to SARS-CoV, the WHO gave the name for it SARS-CoV-2, or in other word, coronavirus sickness, as it was known in 2019 (COVID-19). The COVID-19 sickness often has modest symptoms, but it can occasionally be fatal and cause severe morbidity. A recently discovered coronavirus called SARS-CoV-2 is connected to SARS and MERS (K. L. Hon et al., 2020).

SARS-CoV-2 is a member of the family Coronaviridae's Betacoronavirus genus. The members of this genes are, human coronavirus (HCoV)-HKU1, human coronavirus (HCoV)-OC43, Middle East respiratory disease (MERS) coronavirus (MERS-CoV), and SARS-CoV-1 (Chan et al., 2015). SARS-CoV-2 is included in the Sarbecovirus subgenus of SARS-related coronaviruses, together with the closely related bat coronavirus RaTG13 and SARS-CoV-1 (Zhou et al., 2020).

A membrane surrounds with an average diameter between 75 and 150 nm contains the single-stranded positive-sense RNA from the SARS-CoV-2 genome. Petrosillo stated that due to the spikes of glycoprotein that coat its membrane, coronaviruses resemble crowns (corona is the Latin word for crown or garland).

Over 30 K nucleotides make up the SARS-CoV-2 genome. This virus and the SARS-CoV share more than 85% of their homology (Petrosillo et al., 2020). The spike (S) surface glycoprotein, membrane (M), envelope (E), and nucleocapsid (N) proteins are the four main structural proteins converted by the SARS-CoV-2 viral genome (MA, 2020).

Each patient's COVID-19 sickness presents with a unique set of symptoms, however the most prevalent clinical signs throughout the disease's many phases are fever, exhaustion, cough, expectoration, anorexia, sputum production, and shortness of breath (Xu et al., 2020; Nanshan Chen et al, 2020). Additionally, less frequent symptoms such a sore throat, headache, disorientation, hemoptysis, shortness of breath, and chest tightness have also been seen (Wu & McGoogan, 2020; Han et al., 2020). Minor symptoms like nausea, vomiting, diarrhea, and gastrointestinal complications have also been described (Pan et al., 2020). Children and adults both experienced the same COVID-19 signs and symptoms, albeit often the severity of the symptoms was lower than it was in the adult patients (zhou, 2020; Anandh, 2020).

2.2. SARS-COV-2 Vaccines

To stop SARS-CoV-2 infection in communities throughout the world, vaccination is the most crucial step in managing this global pandemic, in addition to the significance of enforcing strategies for infection prevention and public health to prevent or minimize the spread of SARS-CoV-2. Immune system activation after vaccination results in the development of SARS-CoV-2 neutralizing antibodies (Cascella & Dulebohn, 2022).

The majority of vaccinations work by injecting genetic material that encodes for the glycosylated spike (S) protein, a key stimulator of the host immune response. The kind of nucleic acid utilized and the method of delivery vary (Majumder & Minko, 2021).

2.2.1. The Moderna COVID-19 (Mrna-1273) Vaccine

A messenger RNA (mRNA)-based vaccination against coronavirus illness is the COVID-19 Vaccine Moderna (COVID-19). The body may elicit an immune reaction and store that knowledge in memory immune cells thanks to the host cells are instructed by the mRNA to produce S-antigen protein that is unique to SARS-CoV-2. The study participants who received the full doses of vaccination (2 doses) and had negative baseline SARS-CoV-2 status showed an effectiveness rate of around 94% after an average time break between the two doses of two months. The information examined at this time supports the conclusion that Covid-19 Vaccine Moderna has more known and prospective advantages than known and possible disadvantages (FT filgotinib, 2020).

The recommended schedule of the vaccine doses:

2 doses (100 μg, 0.5 mL each) with a interval of 28 days.

Recommended for age, 18 years of age and above (FT filgotinib, 2020).

2.2.2. The Pfizer BioNTech (BNT162b2) COVID-19 vaccine

A COVID-19 mRNA vaccine is called BNT162b2. A two-dose course of BNT162b2 administered 21 days apart in the randomized vaccination study provided 91% protection (95% Confidence Interval (CI): 89 to 93%). After seven days from the second dose against symptomatic SARS-CoV-2- Based on a

standard follow-up of six months, the patients who are above 16 years old had infection with the ancestral strain (Thomas et al., 2021) . In general, 90 to 100% vaccination effectiveness was seen across subgroups that were classified by age, sex, race, body mass index, and comorbidities. A longer inter-dose interval of 12 weeks increases immunogenicity in terms of neutralizing antibodies (Parry et al., 2021), demonstrating that extended inter-dose intervals will produce a positive immunological response, especially in older persons.

Interval between dose 1 and dose 2:

In comparison to the manufacturer-recommended 3–4-week interval, the efficacy of the vaccine was much stronger against both infection and hospitalization when the period between doses was extended, at 7-8 weeks (Imamura et al., 2021). Compared to a 4-week interval, an inter-dose interval of 8 weeks or longer was linked to a decreased incidence of myocarditis (Buchan et al., 2022).

Recommended Dosages:

SAGE advises giving two doses (30 g, 0.3 ml each), four to eight weeks apart, intramuscularly into the deltoid muscle for all people above the age of 12.

SAGE advises giving two doses of 10 g, 0.2 ml each, intramuscularly into the deltoid muscle, 4 to 8 weeks apart, preferably 8 weeks apart, for children aged 5 to 11 years.

According to the label, a regimen of two doses spaced three weeks apart and a third dosage given at least eight weeks following the second dose is advised for babies and kids between the ages of 6 months and 4 years. Nevertheless, nations can think about extending the time between the first and second doses to

up to 8 weeks (Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine, 2021).

2.2.3. The Valneva VLA2001 COVID-19 Vaccine

The Valneva (VLA2001) vaccine is a complete SARS-CoV-2 virus that has been thoroughly purified, inactivated, and adjuvanted. The whole-virion inactivated vaccine is made to attach to alum with the aid of the toll-like receptor 9 agonist (cytosine phosphor-guanine: CpG 1018) adjuvant (Lazarus et al., 2021; Danon et al., 2020). The two adjuvants boost the vaccination-induced cellular immune response without harming health. The SARS-CoV-2 spike protein and other viral surface antigens stimulate cellular immune responses (Th1) after delivery that are directed against the spike and other viral surface antigens, as well as neutralizing and other functional binding antibodies. These responses are all thought to help protect against COVID-19. Since inactivated vaccines cannot multiply, they cannot spread disease to people (Interim, 2022).

The national regulatory body of Malaysia is now reviewing the vaccination dossier, and submissions have been made to the WHO for emergency use listing (EUL) as well as to the regulatory bodies of Argentina and Thailand. The vaccination will be referred to as VLA2001 in the text that follows (Interim, 2022).

The recommended first vaccination series consists of two intramuscular doses of 0.5 ml each. The second dose is recommended to be administered at least 28 days after the first dose (Interim, 2022).

2.2.4. The Sinovac-CoronaVac COVID-19 vaccine

Another type of vaccination is an inactivated whole virus vaccination and called Sinovac-CoronaVac. It is adjuvanted with aluminum hydroxide. Two doses of Sinovac-CoronaVac, given at intervals of 14 days, showed effectiveness of more than 50% infection against the SARS-CoV-2 symptomatic (95% confidence interval [CI]: 36-62%), 100% (95% CI:17-100%), severe COVID-19 infection of 100% (95% CI:56-100%), and hospitalization of 100% (95% CI:56-100%), starting 14 days following the second dosage. The group that received the vaccination experienced no COVID-19-related fatalities; the placebo group experienced one COVID-19-related death. According to WHO in 2021 The efficacy of the vaccination was maintained in both groups of individuals with and without comorbidities, regardless of past SARS-CoV-2 infection (WHO, 2021).

The initial vaccination series is advised to be administered in also 2 vaccine doses with 0.5 ml for each dose intramuscularly into the deltoid muscle. The vaccination can be given with an interval of 2-4 weeks, according the product label from the manufacturer. The WHO suggests a 4 week interval. If the second dosage is scheduled for longer than 4 weeks, it should be administered as soon as feasible. It is advised that everyone who has been immunized receive two doses (WHO, 2021).

2.2.5. The Oxford/AstraZeneca (ChAdOx1-S [recombinant] vaccine)

Axzevria functions by getting the body ready to fight COVID-19. It is composed of an adenovirus, which has been altered to carry the gene necessary to produce the SARS-CoV-2 spike protein. The COVID-19 virus has a protein on

its surface that is necessary for the virus to penetrate cells in the body (Union et al., 2021).

The vaccine introduces the SARS-CoV-2 gene into body cells after administration. The spike protein will be created by the cells using the gene. The immune system of the individual will then identify this protein as foreign and make antibodies as well as activate T cells, which are white blood cells, to begin attacking it (Union et al., 2021).

The individual's immune system will recognize the SARS-CoV-2 virus and be prepared to fight it off if they subsequently come into contact with it (Union et al., 2021).

Vaccine effectiveness about 20% of the 32 451 study participants in the worldwide phase 3 trial, which was performed in Chile, Peru, and USA, were 65 years or older (Falsey et al., 2021). Asymptomatic SARS-CoV-2 infection was reduced after vaccination 74% of the time (95% confidence interval [CI]: 65.3-80.5%). In the vaccination group, there were no cases of serious or life-threatening illness; in the placebo group, there were 8 cases. The effectiveness of the vaccine in trial participants 65 and older was 83.5% (95% CI: 54.2-94.1%) (Falsey et al., 2021). The background information on the vaccination with AZD1222, which was released on March 1, 2021, has more comprehensive details on the effectiveness and safety of this vaccine (World Health Organization, 2021).

The ChAdOx1-S [recombinant] vaccine has an effectiveness of more than 70% (95% CI: 63-79%) against the symptoms of COVID-19 infection, according to the preliminary data analysis that took interdose interval into account from trial participants who received two doses separated by 4 to 12 weeks (Emary et al.,

2021). When there was a longer time between doses, vaccine effectiveness expected to be higher (World Health Organization (WHO), 2022).

2.2.6 The Novavax Vaccine

Recombinant SARS-CoV-2 spike protein nanoparticles are given as a coformulation with the adjuvant Matrix-M in the Novavax vaccine (NVX-CoV2373). Vaccines made of proteins have been used to prevent diseases including hepatitis B, pertussis, and the human papillomavirus. Matrix-M has been employed in prelicensure trials for a number of ailments as well as research on NVX-CoV2373, despite the fact that it has not yet been included in a licensed vaccine (30 thousand individuals in stage 1 through stage 3 trials) (4 200 recipients total). Matrix-M is added to NVX-CoV2373 to increase its immunogenicity since it encourages the activation of innate immune cells and antigen processing (Reimer et al., 2012).

2.2.7. The Janssen vaccine

The Janssen vaccine (Ad26.COV2-S [recombinant]) protects against coronavirus disease (COVID-19). The SARS-CoV-2 Spike protein gene, which commands the host cells to manufacture this protein, is carried by the vector virus. The generation of antibodies and the formation of memory immune cells, which guard against infection and illness, are triggered by the presence of the spike protein on the surface of the host cell. The Janssen's Vaccine ability against symptomatic SARS-CoV-2 infection was 67% in clinical trials (ENSEMBLE 1), more than 75% against COVID-19 after 14 days and around 85% after 28 days, and finally more than 90% against hospitalizations (AEMPS, 2021). Minor

vaccination efficacy (VE) has been observed, nevertheless, as varieties of concern have emerged. In particular, against symptomatic infections, particularly those caused by SARS-CoV-2 variations of concern, such as the Omicron form, the ENSEMBLE 2 trial and subsequent investigations from South Africa revealed enhanced vaccination effectiveness with two doses of vaccine given 2 months apart. When analyzed at least 14 days following the second vaccination, the primary analysis of ENSEMBLE 2 data reveals that VE against moderate to severe/critical COVID-19 was 75% and VE against severe/critical COVID-19 was 100%. The effectiveness of the vaccination gradually declines over several months, as it does with other COVID-19 shots (AEMPS, 2021).

2.3. Immune Responses to Infection and Vaccination

2.3.1. Immune Responses to Infection

The immune system is composed of chemicals and cells with specific functions for fighting infection. Innate immunity or in other words, natural immunity and adaptive immunity, in other words, acquired immunity and other crucial factors have a role in the immune system's response to invasive infections. Innate immune responses are affected by the production of inflammatory cytokines and chemokines, phagocytic or killer cells, and other variables. T and B cells that are specific for antigens are produced as a result of adaptive immunological reactions. As effector T cells, which release a variety of cytokines or initiate cytolysis to destroy target cells, antigen-primed T cells promote clonal proliferation and differentiation (Medzhitov & Janeway, 1997; Aderem & Ulevitch, 2000; Janeway & Medzhitov, 2002). Immunoglobulins, which B cells secrete and which are in charge of destroying external bacteria. Innate responses

are generated at the borders of sites of microbial penetration, as opposed to secondary lymphoid organs where adaptive immune responses are created, such as the spleen and lymph nodes. Recent research has demonstrated that a connection between the innate and adaptive immune systems is provided by dendritic cells by moving through lymphatic arteries and are essential for the beginning and control of both types of immunity. It was once believed that innate immunity consisted of immunological reactions that were non specific and were characterized by phagocytosis and digesting of bacteria and by neutrophils and macrophages to foreign substances. Innate immunity, on the other hand, is quite specific against microbes and is able to distinguish between pathogens and self (Medzhitov & Janeway, 1997; Aderem & Ulevitch, 2000; Janeway & Medzhitov, 2002).

For innate immune recognition, a few number of pathogen recognition receptors—receptors that are germline-encoded—are required. These receptors search for conserved molecular patterns (CMPs) known as pathogen-associated molecular patterns (PAMPs), and then employ intracellular signalling to produce inflammatory cytokines. The most extensively researched family of pathogen recognition receptors is the toll-like receptor (TLR) family, and members of this family can detect a wide range of PAMPs (Akira et al., 2001).

In order to recognize microbial components, TLRs are crucial. The epithelium's dendritic cells produce TLRs and serve as sentinels as the first line of defense (Jarrossay et al., 2001; Kadowaki et al., 2001; Hornung et al., 2002). It's noteworthy to note that distinct subsets of dendritic cells express different sets of TLRs, and as a result, they play specific roles in innate responses and the formation of various T-cell subsets (Azuma, 2006).

Dendritic cells that have been exposed to antigens change from being highly sensitive endocytic cells to nonendocytic cells, which move to local lymph nodes to provide antigens to immature T cells and are less vulnerable to the peripheral infectious environment. During migration, dendritic cells develop into mature dendritic cells with excellent antigen-presenting capacity by exhibiting high amounts of peptide-MHC and a range of molecular costimulators, such as CD86 and CD40. To activate T cells specific for an antigen in the T-cell zone of lymph nodes, memory T cells collaborate with migratory dendritic cells. To determine how well T-cell immune responses work against infections, CD4+ T cells are crucial. The two subtypes of effector CD4+ T cells are T helper (Th)1 and Th2 (Mosmann & Coffman, 1989). Interleukin-4, -5, -6, and -13 are secreted by Th2 cells and are essential for optimal antibody production as well as the eradication of external microbes like helminths and nematodes, whereas Th1 cells secrete interferon (IFN)-c, tumor necrosis factor (TNF)-a, and TNF-b [lymphotoxin (LT)], which are crucial for the eradication of intracellular infections. In regulating pathogenic immunological reactions, these two T-cell subsets are essential. While some organ-specific autoimmune illnesses and tissue damage have been linked to Th1-mediated immune responses, systemic autoimmune diseases and allergies have been linked to Th2-mediated immune responses (Gately et al., 1998).

According to a study by Gately published in 1998, cytokines, receptor-mediated signal transduction pathways, and costimulatory molecules are all involved in the commitment to the Th subsets. Transcription factors are also a factor. Crucial cytokine interleukin-12 encourages immunological responses mediated by T helper 1 and regulates T helper 2 responses (Gately et al., 1998).

The use of costimulatory molecules during cognate interactions between dendritic cells and T cells may significantly affect the growth of Th1 and Th2 cells (Salomon & Bluestone, 2001). Different dendritic cell subsets are responsible for activating various T-cell responses in response to certain TLRs (Ito et al., 2002).

However, certain dendritic cells instead of inducing immunity, cause T-cell tolerance (Thompson & Thomas, 2002; Moser, 2003; Enk, 2005). Dendritic cells can give antigens but are unable to create the powerful costimulators CD80 and CD86 that encourage T-cell tolerance. This is because they phagocytose the dead cell without receiving any pathogen-related signals. Dendritic cells may become less effective in presenting antigens when anti-inflammatory cytokines like TGF-b and interleukin-10 (IL-10) transmit them tolerogenic signals (Azuma, 2006).

Dendritic cells alone do not regulate the immune system. Some T-cell subsets have the ability to directly impair effector T cell activity. Among these regulatory T cells, the most well-known are the naturally occurring CD4+ CD25+ regulatory T cells, sometimes referred to as natural regulatory T cells (Sakaguchi, 2005). To grow and become activated, CD4+ CD25+ regulatory T cells from the thymus require antigenic stimulation, a lot of interleukin-2, or CD28 costimulation (Salomon et al., 2000).

Regulatory T cells, on the other hand, are anergic and hypoproliferative when they are acting as suppressors. Additionally, regulatory T cells with CD4+ CD25+ release TGF-b and interleukin-10. It appears that while their development and maintenance do need these cytokines and cells that present antigen, their suppressive action does not (Fontenot & Rudensky, 2005). Cell-to-cell interaction may be necessary for regulatory T cells to perform their role. Foxp3, a forkhead

transcription factor, is crucial for the growth and operation of natural regulatory T cells (Fontenot & Rudensky, 2005; Hori et al., 2017). Natural regulatory T cells have recently been shown to be able to induce T-cell tolerance to external, non-self antigens such microbial infections. They had previously only been examined in connection to peripheral self-tolerance and autoimmune (Sakaguchi, 2005).

2.3.2. Immune response to vaccine antigens

Vaccines function by inducing the immune system to react to a virus or bacteria. The immune system develops a "memory" as a result. This immunological memory enables the body to "remember" a particular virus or bacteria so that it can defend itself against it and stop sickness that it may otherwise cause. The majority of vaccinations include a virus or bacteria in weakened, inactivated (killed), or minuscule amounts that cannot spread illness. It is known as an antigen. A person's immune system recognizes the antigen as alien when they get a vaccination (Siegrist & Lambert, 2016).

The immune system's macrophages, T cells, and B cells are among the cells that are stimulated by these antigens. When proteins or other antigens enter the body, macrophages ingest them and break them down into smaller antigens, which trigger an immune response. Some of these fragments are transported to the cell surface by a protein known as MHC (major histocompatibility complex), where they are displayed but remain encased in the cleft of the MHC molecule. T cells are able to detect the pieces of antigen that are presented, which prompts B cells generate antibodies against the fragments in addition to other immune reactions. According to studies, T cells are unable to distinguish between an antigen segment that is particular to an infecting microorganism and an antigen

fragment that is specific to a vaccine because they only identify antigen fragments from proteins predigested by macrophages, claims Berkower (John Elkington, 1997).

The immune system of the person will recall the incident in the event that they later come into touch with the real virus or bacteria. The virus or bacteria can then be promptly eliminated by producing the appropriate antibodies and activating the appropriate immune cells. This shields the individual from the illness (Siegrist & Lambert, 2016).

Which kind of antibodies is produced depends on the cytokines secreted by stimulated immune cells, which come in many different chemical forms. For instance, immunoglobin E (IgE) antibodies, which trigger allergic reactions, can be released by B cells as a result of the cytokine interleukin 4. IgG, which predominates in the blood, or IgA, which predominates in bodily fluids, are the two forms of Ig that B cells preferentially produce in response to certain cytokines (John Elkington, 1997).

The collection of MHC molecules and the genes that control their synthesis varies greatly from person to person. Distinct MHC molecules bind to different antigen fragments. As a result, even if two individuals' immune systems may react to the identical protein in a vaccination, their T cells may accomplish this in many ways (John Elkington, 1997).

The benefits of vaccination extend beyond those who have already gotten them. By lowering the chance of exposure to illness, it also indirectly protects community members who have not received vaccinations, such as small children who are too young to get vaccinations or those with compromised immune systems (Siegrist & Lambert, 2016).

2.4. Breastfeeding

Infants should only consume breast milk since it includes all the nutrients necessary for their health, growth, and development (Kalantari & Haghighian Roudsari, 2013; Ip et al., 2007). Breast milk is the only form of food that should be consumed since it includes a lot of antioxidants that protect infants from viruses (Lönnerdal, 2000; Gill et al., 2007). Antioxidants from breastfeeding, such vitamin C and vitamin E, help to prevent or lessen oxidative damage to numerous human tissues (Li et al., 2009). Breast milk contains a number of anti-inflammatory substances that shield children against the negative effects of inflammation (Hoppu et al., 2005).

The maternal hypothalamus and hormones generated from the pituitary gland control the production of milk. The two primary hormones involved in milk production and the subsequent letting down reaction are oxytocin and prolactin (Uvnäs-Moberg et al., 1990). Before the infant is born, milk production goes through several distinct phases. Colostrum, which is made accessible after childbirth, is the first milk. Pre-colostrum can occasionally be seen before the postpartum period. Colostrum, the newborn's first milk, is abundant in protein, sodium, and immunoglobulins while being minimal in lactose (Żelaźniewicz & Pawłowski, 2018). After 30 to 40 hours after delivery, lactose concentration rises and other components are diluted as milk volume rises (Weaver & Hernandez, 2016).

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Due to the high concentration of immunoglobulins in colostrum, it has powerful immune-stimulating properties (Verd et al., 2018). The phrase "baby's first vaccination" is frequently used. Through the secretory IgA (sIgA), IgM, and IgG, the GI tract develops mucosal immunity. These essential immunoglobulins preserve the gut's barrier and are crucial in the battle against microbes (Dzidic et al., 2018; Toscano et al., 2017).

Up to the first six months of an infant's life, breastfeeding exclusively lowers the risk of gastrointestinal illnesses and asthma, promotes prevention of childhood obesity (Arenz et al., 2004; Koletzko, 2006; Kinsella & Monk, 2012; Shields et al., 2006) and diabetes in later years of childhood (Owen et al., 2012; Gunderson, 2008), and may be linked to lower cholesterol levels (Owen et al., 2012). Additionally, compared to non-breastfed children, breastfed youngsters score higher on mental-cognitive abilities tests (Bernard et al., 2013).

The oligosaccharides and microbiota found in milk, including Bifidobacterium and Lactobacillus, provide the gut an antibacterial effect (Dzidic et al., 2018; Toscano et al., 2017). Additionally, this leads to the creation of crucial minerals such vitamin B12, B6, folate, and vitamin K. Early breastfeeding

will make it easier for the baby's naturally sterile gut to become colonized by these safe microorganisms as well as mother organisms rather than the organisms of the hospital infant room and the other caregivers. Thus, it is advised to move in early and keep the infant with the mother (Telang, 2018).

Lactoferrin, another component, will operate by promoting iron absorption and inhibiting bacterial oxidation of iron. This aids in the growth of bacteria. It provides immunological defense against bacterial, viral, fungal, and pathogens (Telang, 2018).

As a result of the infant's oral germs being exposed to the mammary gland, a retrograde milk flow would result in the creation of antibodies as well as an immune system reaction. By using the mother's immune system and boosting the newborn's immature immune system, the infant would eventually be protected against illnesses by these antibodies (Laouar, 2020).

2.5. Composition Of Breast Milk

Colostrum is the biological fluid that acts as "pre-milk" that is released by the mammary glands of female mammals within the first few days (WHO & UNICEF, 2003; Ahonen et al., 1998; Mosca & Giannì, 2017) of your newborn's existence. The liquid's composition is diverse from that of the milk produced afterwards. It has a golden color, is thick and sticky, is in little amounts, but is very nutritious. It contains a variety of elements that are thought to be crucial for the development of the baby's immune system, including Igs, lactoferrin, growth factors, and lysozyme. All nursing mammals' milk, colostrum, and blood include immunoglobulins, which are blood molecules created by the body's innate immune system and "generated by plasma cells (white blood cells)". In

comparison to many other animals, pregnant humans transfer some amount of passive defense immunity to the fetus to a greater extent. Human colostrum also contributes to the development of neonatal immunity, although in many other mammals it does not provide immunological protection. Numerous studies have shown that colostrum feeding can transport cytokines, Igs, growth factors, antimicrobial substances, and maternal immune cells to the infant (El-Loly, MM, Guirguis, AH and AS Abdel-Ghany, 2018).

Mature human milk has a fat content of 3%–5%, a protein content of 0.8%–0.9%, a lactose content of 6.9%–7.2%, and a mineral content of 0.2% expressed as ash. It contains 60–75 kcal per 100 ml of energy. Compared to mature milk, colostrum has a much higher protein content and a lower carbohydrate content (Jenness, 1979). Although there are significant nocturnal changes and a rise in fat content with each nursing session, during breastfeeding the fat content does not change regularly. Casein that is similar to beta-casein in cow's milk, alpha-lactalbumin, lactoferrin, immunoglobulin IgA, lysozyme, and serum albumin are the main proteins found in human milk. There are also several "minor" proteins and numerous enzymes. Human milk's necessary amino acid composition closely approaches the pattern that has been proven to be best for human newborns. Other than serving as a source of amino acids, the potential particular activities of milk proteins and enzymes are still completely unknown. The main sugar in human milk is lactose, but there are also 30 or more oligosaccharides that range in size from 3- to 14 saccharide units per molecule and all include terminal Gal-(beta 1,4)-Glc (Jenness, 1979). These might total up to 1 g/100 ml in mature milk and 2.5 g/100 ml in colostrum. Due to their capacity to encourage the growth of specific strains of lactobacilli, some of them may work

to regulate the gut flora. In human milk fat, there are significant amounts of palmitic and oleic acids. both of which were substantially concentrated in the 2-position of the triglycerides, with the latter also being heavily concentrated in the 1- and 3-positions. With the nature of the food, milk fat's fatty acid content, and particularly the fatty acids it provides, varies somewhat. Phospholipids comprise phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl serine, phosphatidyl inositol, and sphingomyelin, with an overall concentration of around 75 mg/100 ml. Na, K, Ca, Mg, P, and Cl are the main minerals discovered in human milk. The range of calcium values reported in different research is 25–35 mg/100 ml. The amount of phosphorus is significantly more consistent in milks of most other species, at 13–16 mg/100 ml, although it is also less concentrated compared to calcium and casein (Jenness, 1979).

Breast milk has a wide range of iron, copper, and zinc concentrations. Many other trace elements have been found. In human milk, nonprotein molecules such urea, uric acid, creatine, creatinine, and a significant number of amino acids make up around 25% of the total nitrogen. Taurine and glutamic acid stand out among the latter. Human milk has nutritionally important quantities of all vitamins except K (Jenness, 1979).

2.5.1. Antimicrobial Factors Found In Breast Milk

In addition to cytokines, polyunsaturated fatty acids, immune-stimulating proteins, glycoproteins like lactoferrin, glycated components like mucins, human milk oligosaccharides (HMOs), and extracellular vesicles, breast milk contains additional substances that have been demonstrated to have widespread antibacterial activity. Mother's milk has a wide spectrum of antiviral properties,

which is an astonishing feature of this diversity of chemicals (Chirico et al., 2008).

Small proteins called cytokines have roles in intercellular signaling. An good diversity of cytokines, both pro- and anti-inflammatory in nature, may be found in breast milk. Transforming Growth Factor (TGF), a cytokine involved in inflammation, appears to have a significant immunomodulatory and antiviral impact. TGF- is a helpful regulator of IgA synthesis and passive immunity. Also, some research reveal that moms who experience high levels of microbial stimulation make milk with higher levels of TGF-, indicating that there may be a control to help with the protection of the infant (Gila-Diaz et al., 2019).

Researchers first postulated that the lipids in breast milk, or rather the by products of their digestion, would have a specific antibacterial and antiviral impact over 30 years ago (Dodge & Sagher, 1991). The fatty acids in breast milk have an antiviral effect, according to recent studies. Phospholipid membranes wrap the triacylglycerols that make up the large vesicles that carry the fats in breast milk. The triacylglycerols are broken down by the salivary and stomach lipases in the infant into monoglycerides and free fatty acids. Depending on their length, saturation level, and quantity of active radicals, free fatty acids exhibit potent antimicrobial and antiviral effects (Gardner et al., 2017). The most effective antibacterial compounds appear to be long-chain polyunsaturated fatty acids, which are effective against both bacteria and viruses (Morniroli et al., 2021).

Lactoferrin from human milk has long been recognized for its beneficial regulation of iron absorption and its antibacterial properties. This protein's capacity to attach to milk's iron and remove it from the bacteria's metabolic

process helps prevent infections from spreading out of control (Habib et al., 2020). A few DNA and RNA viruses that Lactoferrin has a powerful antiviral impact on by preventing viral entrance into host cells include Rotavirus, Human Immunodeficiency Virus, Human Papillomavirus, and Herpes Simplex Virus (Kell et al., 2020).

There are two ways that this antiviral impact is implemented. The virus may be read immediately by lactoferrin, which stops it from attaching to the cell. Secondly, heparan-sulfate molecules, which are present in the extracellular matrix as well as attached to cell membranes, can be bound by lactoferrin and occupy their binding sites. To make it easier for viruses to connect to cell receptors, many of them exploit them as attachment and concentration sites (Kell et al., 2020).

Large glycosylated molecules known as mucins may be found in many different biological fluids. Body fluids have a viscosity that is increased with their presence due to their unique structure. Types 1 and 4 of the different mucin subtypes (MUC1 and MUC4) are detected in breast milk. These subtypes have shown antiviral efficacy in vitro against the HIV virus, the influenza virus, and other viruses (Mall et al., 2017).

The unconjugated carbohydrates known as human milk oligosaccharides (HMO) have a dual role in the newborn's immune system's growth. HMOs encourage the growth of a beneficial microbiota, which has significant implications for immunity (Tlaskalová-Hogenová et al., 2020). It is now understood that these substances are crucial prebiotics that support the growth of bacterial strains that are beneficial to health, feed the newborn microbiota, and indirectly modulate the immune system. Yet, research in recent years has also

indicated that they have a direct antiviral purpose. Rotavirus, HIV, norovirus, and influenza viruses are just a few of the viruses that prevent some viruses from connecting to the host cell in HMOs (Morozov et al., 2018). HMOs function as the mucosal surface's soluble decoys, attaching the virus to themselves and preventing it from entering the cell because they share the mucosal surface's glucosidic structure (Morniroli et al., 2021).

2.5.1.1. k-Casein

By functioning as a soluble sensor analogue of the membranes of epithelial cells, K-casein, a small casein protein present in breast milk, inhibits Helicobacter pylori from adhering to the human stomach mucosa (Lönnerdal, 2003).

2.5.1.2. α-Lactalbumin

Several casein and -lactalbumin hydrolysis compounds have been shown to have antibacterial activity against C. albicans, Klebsiella pneumoniae, staphylococcus, and streptococcus (Pellegrini et al., 1999). Human milk contains -lactalbumin, which also has the ability to bind to oleic acid. As a result of this conformational shift and the release of Ca2+ ions, HAMLET (human -lactalbumin rendered deadly to tumor cells) is created (Svanborg et al., 2008).

2.5.1.3. Haptocorrin

Several ideas contend that the haptocorrin protein in breast milk has the ability to bind to vitamin B12 and so inhibit the growth of microorganisms. Haptocorrin. coli has been demonstrated to be resistant to a strain of

enteropathogenic E at comparable levels when exposed to digestive enzymes and when left undigested, indicating that this protein assists in the defense against infections in nursing infants (Adkins & Lönnerdal, 2003).

2.5.1.4. Osteoprotegerin

Compared to human blood, osteoprotegerin levels in human milk and mammary epithelial cells can be up to 1,000 times higher (Vidal, Van Den Broek, et al., 2004). Considering its capacity to bind to the TNF-related apoptosis-inducing ligand (TRAIL) and trigger caspase-dependent apoptosis, especially in Th1 cells, it is assumed to be essential for regulating the balance of Th1/Th2 during the development of newborns' immune systems (Vidal, Serrant, et al., 2004).

2.5.1.5. Soluble CD14 (sCD14)

The concentration of the sCD14 molecule in colostrum and breast milk is higher than that in serum by more than 20 times (Hosea Blewett et al., 2008). When bacteria colonize the gut, this factor affects how innate and adaptive immune responses are controlled, which in turn controls intestinal homeostasis in infants (Labéta et al., 2000).

2.6. Immunoglobulins

A history of the mother's antigen exposure and immune system response may be seen in the immunoglobulins found in breast secretions, which come from several sources. When a baby is sucking, the milk ejects immunoglobulins from the mammary gland through receptor-mediated pathways through the mammary

epithelial cells. The newborn's gastrointestinal system is then exposed to the immunoglobulins. The immunoglobulins are stable enough to provide the newborn with protective benefits even though the environment is primarily created for digestion to obtain nutritional benefits. This may be done by absorbing into the neonatal vascular systems of some species or by engaging in immunological activity in the digestive tract (Hurley & Theil, 2011).

IgA, IgM, and IgG are the three major classes of the human Igs family, whereas IgD and IgE are the two minor groups. IgG, IgM, and IgA are the three primary classes found in both bovine and human milk. When compared to blood, the varied Ig fraction levels in colostrum and milk vary dramatically by species and are frequently unmatched. For instance, the IgA class makes up around 90% and 15% to 20% of all Igs in human colostrum, milk, and blood, respectively (El-Loly, MM, Guirguis, AH and AS Abdel-Ghany, 2018). In cattle, Igs are divided into four isotypes according on the amount of heavy chain they contain: IgG (IgG1 and IgG2), IgA, IgM, and IgE (El-Loly, MM, Guirguis, AH and AS Abdel-Ghany, 2018). High concentrations of IgG, IgA, and IgM molecules can be seen in milk, more so in colostrum. IgG predominates in colostrum, milk, and blood, accounting for around 80–90, 60–70, and 90% of all Igs, respectively, while IgA is the main Ig class in human milk (Mix et al., 2006; Zhao et al., 2010).

Immunoglobulin IgA migrates to mucosal regions and produces local sIgA antibody responses. A molecules are generated by B lymphocytes of the peripheral immune system in the mammary gland and they are represented in maternal Peyer's patch lymphoid cells (Hanson et al., 1994). The immune system of neonates is not fully developed for the first 5 to 6 months of life because the mucosal surfaces of their respiratory and gastrointestinal tracts lack antibodies

.After delivery, secretory IgA levels in breast milk are still high for at least 7.5 months, and they are crucial for maintaining the passive humoral response (Kompaneets et al., 2020).

Infants who are exclusively breastfed getting around 0.3 g/kg/day of SIgA, which accounts for more than 80 % of the overall immunoglobulins in human milk. The majority of this protein's action is local since only approximately 10% of the intestines absorb it and send it to the bloodstream (Brandtzaeg, 2010; Drummond & Howe, 2001). SIgA and, to a lesser extent, SIgM form the infant's initial line of defense against foreign antigens in the gut. Due to this, SIgA molecules continue to function throughout the infant's digestive system and affect the binding of commensal or pathogenic microbes, poisons, viruses, and other antigenic substances, such as lipopolysaccharide (LPS), inhibiting their adherence and penetration into the epithelium without inducing inflammatory reactions that may harm the infant.. Immune exclusion refers to this procedure. High-affinity pentameric and dimeric IgM and IgA antibodies carried by the pIgR may even inactivate viruses (like influenza and rotavirus) inside epithelial cells to transfer pathogens and their byproducts back into the lumen and lessen cytolytic damage to the epithelium (Johansen & Brandtzaeg, 2004). Since they function as a backup for the absence of SIgA in mucous membranes and are prevalent in these secretions, SIgM antibodies are essential in persons with selective IgA deficiency (Palmeira et al., 2009).

IgM antibodies, the second most common immunoglobulin, may be found in human breastmilk at concentrations of up to 2.5 mg/mL. High avidity IgM antibodies that are reactive with viruses and bacteria may provide considerable protection for the mucosal surfaces of neonates. IgG's opsonizing activity can

have a neutralizing effect as well as the capacity to activate the complement system and cause antibody-dependent cytotoxicity. Nevertheless, it is thought that newborns' mucosal surfaces do not exhibit these characteristics to their full extent. A little quantity of IgG, or 0.1 mg/mL (10% of serum levels), can be found in human milk (Hanson et al., 2003).

2.7. Lactoferrin

First found in cow's milk, lactoferrin is an iron-binding glycoprotein that was subsequently found in human milk. Large amounts of lactoferrin are found in milk, tears, saliva, seminal fluid, and certain white blood cells, among other mammalian secretions (REDDY et al., 1977).

The observed lactoferrin concentrations in human milk and colostrum are 2 to 4 grams/liter and 6 to 8 grams/liter, respectively. Lactoferrin is only partially saturated with iron in its normal condition (5 to 30 percent). Many biological functions of lactoferrin have been hypothesized, including antibacterial and anti-inflammatory properties, protection against gastrointestinal infections, provision of an iron-binding antioxidant protein in tissues, collaboration with certain immunoglobulins and other protective proteins, and potential encouragement of the proliferation of animal cells like lymphocytes and intestinal cells are all examples of its participation in local secretory immune systems. Long hypothesized but unconfirmed, milk lactoferrin may play a part in the intestine's absorption of iron (REDDY et al., 1977).

The majority of microbes require iron for development, and lactoferrin may prevent or even stop the growth of germs by depriving them of iron. The organism's need for iron, exogenous iron is accessible, the quantity and saturation

of lactoferrin with iron, and other factors all affect how efficient lactoferrin's antibacterial activity is. (REDDY et al., 1977).

Lf has two distinct pathways that contribute to its antibacterial properties. Its main function is to bind free iron, eliminating a crucial substrate for bacterial development and having a bacteriostatic effect. The second method entails the Lf coming into direct contact with the infectious agent. When Lf attaches to the lipopolysaccharide in bacterial walls, it may potentially harm the bacteria by causing the generation of peroxides, which are catalyzed by Lf-bound iron (III) ions and cause bacterial cell lysis (Giansanti et al., 2016).

2.8. Lactoperoxidase

Heme-containing lactoperoxidase (LPO) is an enzyme that belongs to the mammalian peroxidase superfamily (Nichol et al., 1987). It is a 595 amino acid protein with an 80-kDa molecular weight that is glycosylated and structured into 20 -helices and two antiparallel -strands (Sheikh et al., 2017).

Peroxidase enzymes are capable of destroying bacteria via oxidative processes. Saliva, milk, tears, and other exocrine gland secretions all include peroxidase activity, as do those from the bronchial, nasal, and intestinal linings. Milk peroxidase, also known as lactoperoxidase, is a well-known enzyme that assists in defense against microbial invasion of the mammary gland and is one of the non-immunoglobulin protective proteins. There are one iron atom per lactoperoxidase molecule (REDDY et al., 1977).

No antimicrobial action exists in lactoperoxidase by itself. Referred regarded as the lactoperoxidase system, which also includes hydrogen peroxide and thiocyanate, is a strong natural antibacterial mechanism. At animal and

human tissues, thiocyanate and hydrogen peroxide are both naturally present, but often in extremely low amounts. The mechanism by which the lactoperoxidase system exerts its antibacterial activity is the reaction between hydrogen peroxide and thiocyanate, which is catalyzed by lactoperoxidase, and produces short-lived hypothiocyanate, which is recognized as a primary antibacterial agent. The lactoperoxidase system's antibacterial activity is based on the suppression of crucial bacterial metabolic enzymes caused by their oxidation by hypothiocyanate (REDDY et al., 1977).

2.9. Lysozyme

In breast milk, lysozyme is a significant, non-specific host defense component. All mammals generate lysozyme, a naturally occurring antimicrobial enzyme that lyses a specific link in the peptidoglycan layer of bacterial cell walls, leading to cell lysis and the bacterium's eventual eradication. Milk, saliva, and tears all generate lysozyme (Masschalck & Michiels, 2003). Lysozyme is one of the components that, together with secretory IgA and lactoferrin, makes milk intake related with nonspecific immunity (Village, 2005; Le Hurou-Luron et al., 2010).

Lysozyme and lactoferrin are two of the most important enzymes found in colostrum. An enzyme called lysozyme may break down the outer layer of Gram-positive bacteria by hydrolyzing the -1,4 bonds in the N-acetylglucosamine and N-acetylmuramic acid residues (Drummond & Howe, 2001). When used in conjunction with lactoferrin, which binds to and removes the lipopolysaccharide in the outer layer of the bacterial membrane, lysozyme can penetrate the interior proteoglycan matrix of the membrane and harm it, killing the bacterium. Gram-

negative bacteria in a test tube can be eliminated by lysozyme (Ellison & Giehl, 1991). Lysozyme is furthermore thought to have antiviral effects (Lee-Huang et al., 1999).

Antibacterial action exists in lysozyme against several microorganisms. Normally, this enzyme works in conjunction with immunoglobulin A or lactoferrin. With immunoglobulin A, lysozyme is efficient against Escherichia coli. When combined with the modest quantities of ascorbate and peroxide found in milk, it lyses several kinds of salmonellae. Lysozyme's anti-Escherichia coli action can be decreased by microwave irradiation. Additionally, lysozyme may have anti-inflammatory properties and can restrict neutrophil migration into injured tissue (REDDY et al., 1977).

2.9. How Breast Milk Could Pass Along Covid 19 Immunity To Infants

Human milk antibodies are a reflection of mucosa-associated lymphoid tissue (MALT) activation that is specific to an antigen in the colon and the airways. Every immunoglobulin isotype seen in human colostrum and milk is present, with Immunoglobulin A (IgA) having the greatest amounts (representing 80% to 90% of all immunoglobulins in human milk), followed by IgM and IgG, the latter of which has low concentrations. It has been widely established that SIgA in breast milk is specific for a number of prevalent respiratory and gastrointestinal bacteria (Goldman, 1993).

IgA can exist in monomeric, dimeric, or trimeric forms; the monomeric form predominates in serum while the polymeric form predominates in secretions, where it is known as secretory IgA (SIgA) (Brandtzaeg, 2010).

Because the infant's adaptive immune response needs time to arrange its architecture and provide protective immunity, the neonatal immune system is regarded as immature (Dowling & Levy, 2014). Therefore, during the first few weeks after delivery, the baby is passively shielded by maternal immunoglobulins delivered during the third trimester of pregnancy, via the placenta (Cinicola et al., 2021). Breastmilk contains maternal secretory immunoglobulin A (sIgA) antibodies, which are mostly obtained from the respiratory and intestinal mucosal immune systems of the mother, continuing passive protection after delivery (Brandtzaeg, 2003).

Because of the secretory IgA antibodies it contains as well as the abundance of other bioactive components, human milk is a secretion that serves the dual purpose of feeding the nursing infant and protecting it from respiratory and enteric illnesses. During breastfeeding, plasma cells originating from B lymphocytes that migrate from different mucosae to the mammary gland, notably from the digestive and respiratory tracts, create specific IgA antibodies locally (Zheng et al., 2022).

Human milk contains SIgA antibodies that are reactive with a variety of pathogens that the mother has encountered during her life, demonstrating her immunological memory built up over time. In the lactation period, plasma cells originating from B lymphocytes move from various mucosae to the mammary gland, particularly from the intestinal and respiratory tracts, and create the SIgA that is found in milk locally. As a result, colostrum often has larger amounts of antibodies than serum does that are reactive with antigens from bacteria that pass through the mucosae (Zheng et al., 2022).

IgA from human milk offers essential antimicrobial protection. harmful microorganisms on the newborn gastrointestinal tract are inhibited clings to the mucosal surface, eliminates microbial poisons, and offers passive protection. IgA is mostly generated in the lamina propria next to the mucosa. Secretory immunoglobulin (sIgA) traps harmful bacteria in the mucus and activates intestinal cilia, acting directly on the mucosal surface by preventing germs from adhering to the host epithelial cell receptors. Additionally, SARS-CoV-2 can be neutralized by sIgA before to binding to and infecting epithelial cells. This method enables sIgA breast milk to offer defense against the SARS-CoV-2 virus getting into the mucosal membrane of the airways (Yin Xia et al., 2020).

During the last trimesters of pregnancy, specific maternal antibodies to SARS-CoV-2 are passed through the placenta and are detectable in the serum of newborns delivered to women who were either naturally infected or who had received a vaccination (Egerup et al., 2021; Flannery et al., 2021). In a similar manner, maternal sIgA specific for SARS-CoV-2 is discovered in the breastmilk of women who either had COVID-19 or had the vaccination (Pace et al., 2021; Polack et al., 2020). As a result, in the case of COVID-19, as in the case of many other diseases, the mother makes use of her reservoir of antibodies to assist the infant in moving from a state of maternal immunologic reliance to one of immunologic self-sufficiency (Conti et al., 2021).

Numerous advantages of breastfeeding have been demonstrated for children. Antibodies in circulation can permeate breast milk and transfer to children, providing passive immunity. Newborns will rely on the mother's passive immunity. This claim serves as the foundation for various investigations looking

at the effects of feeding breast milk tainted with COVID-19 on the wellbeing of neonates (Aiman et al., 2020).

CHAPTER III

Methodology

3.1. MATERIAL AND METHODS

3.1.1. Tools and Equipment

The contents of the test kit

Antigen-coated microplate wells

Calibrator (IgA, human)

Positive control (IgA, human)

Negative control (IgA, human)

Enzyme conjugate (peroxidase labeled anti-human IgA)

Wash buffer

Chromogen/substrate solution (TMB/H2O2)

Stopping solution (0.5 M sulfuric acid)

3.1.2. Additional Materials and Equipment

EUROLabWorkstation ELISA

Automatic microplate washer

Microplate reader

Calibrated pipettes

Pipette tips

Incubator

Eppendorf tubes

3.3. Kit

Anti-Sars-Cov2 ELISA (IgA) kit

3.3.1.Purpose of usage:

In order to assist the diagnosis of SARS-Cov-2 infection and to complement direct pathogen detection, enzyme immunoassay (ELISA) allows semiquantitative in vitro detection of immunoglobulin IgA class antibodies targeting SARS-Cov-2 in serum, EDTA, heparin, or citrate plasma.

Detection of IgA antibodies is suitable for monitoring the development of the immune response after positive direct pathogen detection.

This product has been specially designed for trading on the EUROLabWorkstation ELISA.

3.3.2. Test principle

The test kit includes microplate strips with eight breakable wells that are individually coated with the SARS-COV-2 spike protein's recombinant S1 domain.

Diluted patient samples are incubated in the wells during the first stage of the reaction. Specific IgA antibodies that bind to antigens are present in positive samples. An enzyme-labeled anti-human IgA (enzyme conjugate), which catalyzes the color reaction, is used in a second incubation to identify binding antibodies.

3.3.3. Storage and stability

The test kit should be stored between +2°C and +8°C , not frozen.

3.3.4. Usage stability

If maintained between +2°C and +8°C and kept clean after the initial use, the reagents are stable until the specified expiration date, unless otherwise noted.

3.4. Sample collection

30 ml of breast milk collected from each of 23 patients in the North Cyprus Near East University Hospital was included in this investigation.

The breast milk was stored at a temperature of -20°C after collection.

3.5. Methods

3.5.1. Analysis Sars-Cov 2 Specific sIgA Titres in Human Milk

For the quantitative evaluation of SARS-CoV-2 IgA class antibodies, a recombinant S1 antigen of SARS-CoV-2 Spike protein-coated anti-SARS-CoV-2 ELISA (IgA) kit was utilized (Euroimmun, Lubeck, Germany). Before analysis, serum samples were diluted with sample buffer in a ratio of 1:101 as per the manufacturer's instructions.

The reagents in the KIT should, as a protocol, be warmed to room temperature (+18 to +25°C) prior to application. Aliquating human milk into eppendorf tubes, which were then centrifuged at 500 g for 15 minutes at 4 °C, was done. After being removed from the fat layer, the aqueous layer was put in a pristine tube. The following step was a 15-minute, 3000 g, 4 °C centrifugation of this aqueous layer.

Figure 1 First centrifuge

Figure 2. Second centrifuge

The last watery layer was taken off and kept at -20°C. First, 10 µL human serum and 1 µL buffer mixture are prepared in clean tubes. 10 µL of each centrifuged breast milk is taken and added to the diluted mixture. 100 µL of calibrator, 100 µL of positive control and 100 µL of negative control are pipetted into the microplate wells. 100 µL diluted patient mixture was transferred to each wells. Incubation at +37°C for 60 minutes. After incubation, the washing step is started. Washing process is performed automatically 3 times. 100 µL of enzyme

conjugate (peroxidase labeled anti-human IgA) is pipetted into each well. Incubate at 37°C for half and hour. 100 µL of chromogen substrate solution is pipetted into each well. Incubation was performed at room temperature for 30 minutes in a dark place. 100 µL of stop solution is added to each well. Color change is observed. At the conclusion of the investigation, each well's absorbance was measured spectrophotometrically at 450 nm. By dividing the optical density of the sample by the optical density of the calibrator, the concentration of anti-SARS-CoV-2 IgA antibodies was determined. The cut off value was taken as 0.8. Samples below this ratio of were evaluated as negative.

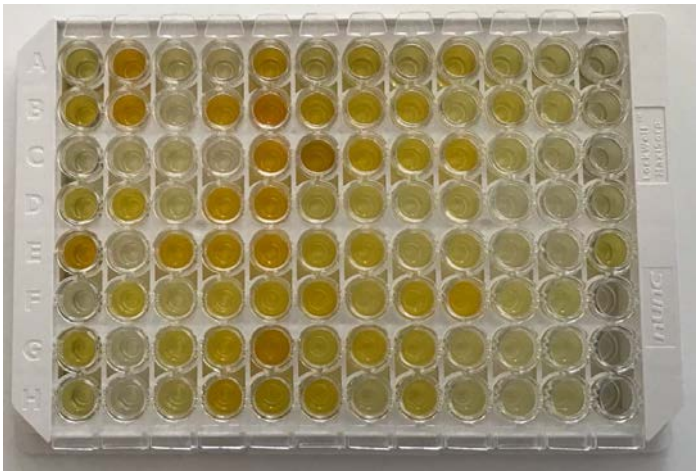

Figure 3. Spectrophotometrical analysis of milk SIgA at 450 nm

3.5.2. Analysis of Sars-Cov 2 Specific IgG Titres in Serum

For serum IgG evaluation, blood samples of approximately 5 mL were taken from each of the volunteers and placed into jelled dry tubes. After being taken, the blood samples were immediately delivered to the laboratory of Near East University (NEU) Hospital, and their serums were separated by centrifugation. The serum samples were stored at -80°C until the time of use. The

sera were tested for anti-S1-RBD IgG using fully automated ELISA (Abbott, Architect i1000sr, Germany) with SARS-CoV-2 IgG II Quant (Abbott, Germany). According to the manufacturer's instructions, a cut-off result of >50 AU/mL is regarded as positive. The assay has a sensitivity/PPA of 92.11, a specificity NPA of 99.97, and it agrees with neutralization in microneutralization assays. 100% PPA and 95.72 NPA.

3.5.3. Statistical Analysis

Quantitative and qualitative variables both have descriptive statistics (frequency and percentage) generated for them (arithmetic mean, standard deviation, median, minimum, and maximum). Hypothesis tests were performed to compare two independent categories for quantitative variables with a non-parametric Mann-Whitney U test since the parametric assumptions were not met. Pearson Chi-Square or Fisher's Exact test was used to investigate the possible associations between qualitative variables, where appropriate.

Statistical analysis and calculations were carried out with SPSS software (Version 26.0 for Mac) while graphical representations were performed with GraphPad Prism (Version 9.0 for Mac). The level of significance was accepted as 0.05 throughout the study.

CHAPTER IV

Findings and Discussion

4.1. Demographic Characteristics of Participants

The age range of 23 lactating women who were included in our study was 25-39 years. Among those 23 paticipants, 4(17.4%) of them were vaccinated with CoronaVac and 19(82.6%) were vaccinated with Pfizer/BioNTech.

History of allergic reactions, autoimmune diseases and having covid-19 infections were found to be 17.4%, 8.4%, and 17.4% respectively. The use of corticosteroids and antibiotics 3 months prior to Covid-19 vaccination was noted as 4.3% and 34.8% respectively. Demographic characteristics of participants of those 23 lactating women and also infants (range 1-21 months) were presented in Table 1.

Table 1. Demographic and clinical characteristics of 23 lactating women and their Infants

Maternal data Age Mean ±SD Median (minimum, maximum) years	31.95±4.42 years 31.00(25-39)
CoronaVac (n,%)	4 (17.4)
Pfizer/BioNTech (n,%)	19(82.6)
History of Covid-19	4 (17.4%)
Allergy	4 (17.4%)
Autoimmune Disease	2(8.7%)
Using Corticosteroids	1(4.3%)
Using Antibiotics 3 months before vaccination	8(34.8%)
The time between the last vaccine dose and the sample taken	3.30±3.44 2.00(0.00-13.00)

4.2. Antibody Titers Among Lactating Women

When the antibody titres were compared according to vaccination group, for CoronaVac, the mean value of serum IgG levels was 726.25±1114.72 and milk sIgA level was 0.24±0.13. While for the Pfizer/BioNTech group, they were 20408.59±14038.00 and 1.84±2.17, respectively. There was statistically difference in both serum IgG titres (p=0.003) and milk sIgA (p=0.038) (Figure 6).

The mean value of serum IgG titres and milk sIgA level for participants who had covid-19 infection was 38423.8 ±3152.4 and 4.62±2.62, respectively. The mean value of serum IgG titres and milk sIgA level for the participants who

had not have covid-19 infection was 12472.26±11962.35 and 0.92±1.23, respectively. There was statistically significant differences for both serum IgG titres (p=0.004) and milk sIgA (p=0.009) (Figure 7).

For the presence of allergy and the use of antibiotics 3 months prior to vaccination, there was no statistical difference for serum IgG and milk sIgA (p> 0.005) (Table 2).

Table 2. Antibody titers among lactating women

		IgG in Serum (AU/mL)		P	SIgA in Milk (Ratio		p
		Mean ±SD	Median (min-max)		Mean ±SD	Median (min-max)	
Vaccine	CoronaVac	726.25±1114.72	256.85 (5.40-2385.90)	0.003	0.24±0.13	0.24 (0.10-0.40)	0.038
	Pfizer/BioNTech	20408.59±14038.00	23282.9 (473.90-40000)		1.84±2.17	0.70 (0.10-6.20)	
History of Covid-19	Present	38423.8 ±3152.4	40000 (33695.2-40000)	0.004	4.62±2.62	5.80(0.70-6.20)	0.009
	Absent	12472.26±11962.35	12056.9(5.40-40000)		0.92±1.23	0.40(0.10-5.20)	
Allergy	Present	22259.82±12871.70	18491.2 (12056.9-40000)	0.35	0.95±0.70	0.90 (0.30-1.70)	0.84
	Absent	15875.21±15273.89	13027.1 (5.40-40000)		1.69±2.23	0.50 (0.10-6.20)	
Use of Antibiotics	Present	9689.12±15741.2	1486.05 (175.90-40000)	0.065	1.58±2.55	0.25 (0.10-6.20)	0.128
	Absent	20877.02±13211.03	23282.9(5.40-40000)		1.56±1.84	0.70 (0.10-5.90)	

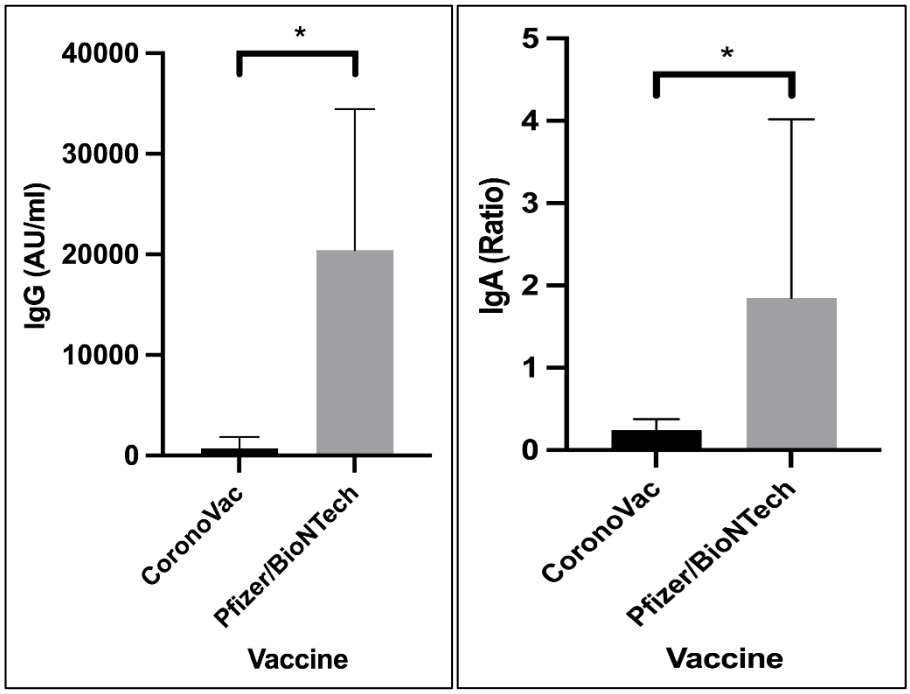

Figure 4. Serum IgG and Milk sIgA titre comparison between vaccine groups

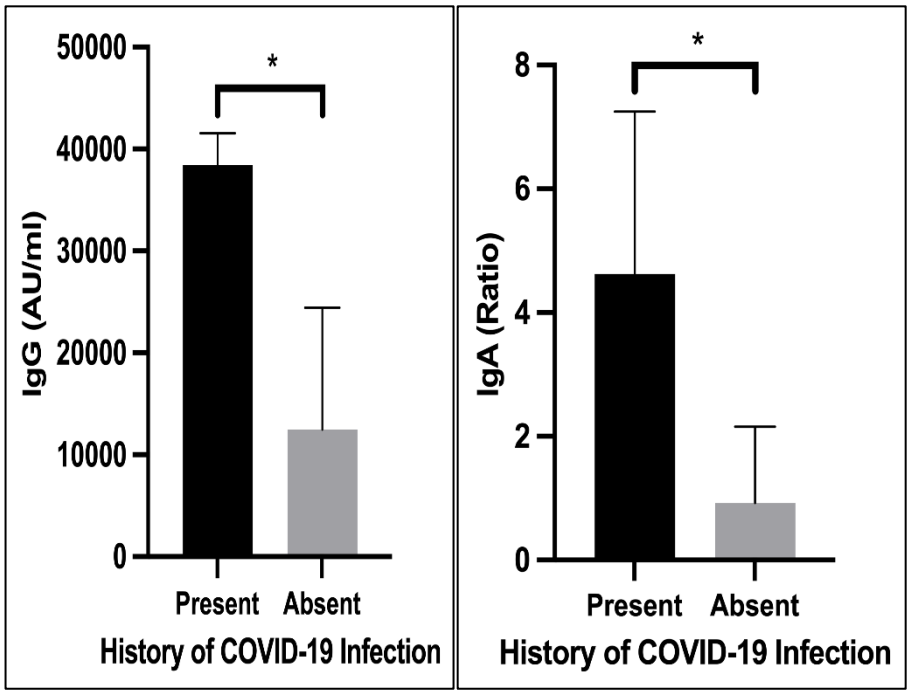

Figure 5. Serum IgG and Milk sIgA titre comparison between history of COVID-19 infection group

According to cut-off value of serum IgG and milk sIgA, participants were divided into negative and positive categories. For vaccination groups, history of covid-19 infection, presence/absence of the allergy and the use of antibiotic use 3 months prior to vaccination, there were no statistically significant differences either for the serum IgG or the milk sIgA (p>0.005) (Table 3).

Table 3. Positivity and Negativity of lactating women according to cut-off value of IgG and Milk sIgA levels

		IgG in Serum (AU/mL)		P	SIgA in Milk (Ratio)		p
		Positive (n,%)	Negative (n,%)		Positive (n,%)	Negative (n,%)	
Vaccine	CoronaVac	3(75)	1(25)	0.170	0(0)	4(100)	0.130
	Pfizer/BioNTech	19(100)	0(0)		9(47.4)	10(52.6)	
History of Covid-19	Present	4(100)	0(0)	1.000	3(75)	1(25)	0.260
	Absent	18(94.7)	1(5.3)		6(68.4)	13(31.6)	
Allergy	Present	4(100)	0(0)	1.000	2(50)	2(50)	1.000
	Absent	18(94.7)	1(5.3)		7(36.8)	12(63.2)	
Use of Antibiotics	Present	8(100)	0(0)	1.000	2(25)	6(75)	0.400
	Absent	14(93.3)	1(6.7)		7(46.7)	8(53.3)	

CHAPTER V

Discussion

Infants rely on their mothers' passive immunity for protection during the first few months of life since their immune systems are still developing and are unable to discriminate between commensal and pathogenic bacteria (Atyeo & Alter, 2021). To establish systemic immunity that gives protection throughout an infant's first several months, particular maternal IgG antibodies are transmitted from the mother through the placenta to the foetal circulation during pregnancy. During the first year of life, maternally produced antibodies gradually decline as the newborn develops protective immune responses as a result of immunisation and early pathogen exposure (Hunagund et al., 2022). After giving birth, nursing moms continue to transmit on to their infant milk-derived antibodies that offer passive mucosal immunity. Human milk contains oligosaccharides, immune system-protecting antibodies such maternal secretory IgA (SIgA), secretory IgM (SIgM), and IgG, as well as other immunologic substances like immune cells, cytokines, glycoproteins (like lactoferrin), immune cells, and other immunologic components (Atyeo & Alter, 2021; Andreas et al., 2015; Hanson et al., 2003).

Although breastfeeding is recommended as the healthiest nutrition source for an infant's first few weeks of life, there were no clear recommendations for nursing mothers at the beginning of the epidemic. After COVID-19 sickness, the existence of IgG and other specific antibodies against SARS-CoV-2, including IgA, in blood and breast milk has been thoroughly examined (Demers-Mathieu et al., 2021).

Research have revealed that during and after acute infection during the global spread of SARS-CoV-2, the cause of coronavirus sickness, the milk

produced by infected mothers had measurable amounts of anti-SARS-CoV-2 IgA and IgG (COVID-19) (Pace et al., 2021). This emphasises the value of immunisation as pregnant women produce a lot of antibodies in response to vaccinations (Pullen et al., 2021; Al-kuraishy et al., 2021).

In our study, a total of 23 lactating women who received the Covid-19 vaccine. The age range of the women included in the study was 31.95±4.42 years, and the infants were7.26±5.51 months. 19 of these women had Pfizer/BioNTech, 4 had Coronavac vaccine, and 4 people had Kovid before vaccination. The ELISA study showed that IgA and IgG levels increased in breast milk and serum.

In the Hospital Universitario Nuestra Seora de Candelaria, a prospective study involving 122 HCWs was carried out from February to April 2021. 14 days after receiving two doses of either the BNT162b2 mRNA (94%) or mRNA-1273 (6%) vaccination, the 98 recipients of the COVID-19 vaccine performed serum and human milk (HM) testing. The mean concentration of SARS-CoV-2 RBD-S1 IgG serum was 3379.64 binding antibody units (BAUs)/mL, but the neutralizing antibody titer was >560.9 BAUs/mL. Those who got the immunization had anti-SARS-CoV-2 RBD-S1 IgG levels that were 12.19 BAUs/mL on average as opposed to 0.02 BAUs/mL in those who did not (P.001). Anti-SARS-CoV-2 S1 IgA antibodies were present in 89% of the HM from immunized women (Ramirez et al., 2021a). A prospective study of breastfeeding women who had received the COVID-19 vaccine and SARS-CoV-2 immunization in Spain comprised 86 nursing moms. The babies were 12.7 months old and the research subjects were 34.6 3.7 years old (mean SD) (mean SD).

Adenovirus-vectored vaccinations (ChAdOx1 nCoV-19, Oxford/AstraZeneca) or mRNA vaccines (BNT162b2 mRNA, BioNTech/Pfizer

and mRNA-1273, Moderna) were given to participants in two doses. IgG and IgA antibodies reacted strongly to the vaccine, especially after the second dosage. IgG and IgA levels rose in comparison to the pre-pandemic population and the baseline time-point. Moreover, it was shown that vaccination increased the levels of IgG and IgA in milk samples (p 0.0001 and p 0.0001, respectively) (Selma-Royo et al., 2022).

Investigations by Juncker et al. were conducted to ascertain the impact of immunization on SARS-CoV-2-specific IgA levels in breast milk (n = 26). They observed an enhanced IgA antibody response and a specific antibody response to SARS-CoV-2 in breast milk following the second BNT162b2 dose (Juncker et al., 2021). According to Perl et al's research (n = 84), the mean levels of anti-SARS-CoV-2-specific IgA antibodies had significantly increased two weeks (p 0.001) and one week after the first treatment, respectively. (BNT162b2). After remaining low for the first three weeks, the mean levels of IgG antibodies specific for the SARS-CoV-2 virus increased at week four (p = 0.004). (Polack et al., 2020).

110 nursing mothers participated in a cross-sectional study in Northern Spain that demonstrated the existence of IgG and IgA isotype antibodies specific to SARS-CoV-2 antigens in serum and milk in nursing women with various SARS-CoV-2 vaccinations. mRNA-1273 was given twice to 20 women (18.2%), BNT162b2 was given twice to 20 women (18.2%), and ChAdOx1-S was given once to 70 women (63.6%). As a result, nursing mothers exposed their children to SARS-CoV-2 protein S IgA and IgG isotype antibodies (Lechosa-Muñiz et al., 2021).

In cord blood and infant follow-up blood tests for mothers who got the vaccination during pregnancy, detectable anti-SARS-CoV-2 IgG antibodies were found. This indicates that the placenta transferred the IgG antibodies to the foetal circulation (Prahl et al., 2022). However, immunisation with COVID-19 during breastfeeding and human milk was contaminated with anti-SARS-CoV-2 antibodies in both cases of pregnancy (Golan et al., 2021; Rosenberg-Friedman et al., 2021; Young et al., 2022).

As little is known about nursing and COVID-19 vaccination, this study's novelty as a topic contributes to one of its strengths. Studies on vaccination and immune response in this population were extremely important because breastfeeding has so many benefits for the newborn and child. The length of breastfeeding and greater levels of antibodies in the mother's milk are being compared for the first time in this study.

Limitations include the inability to rule out prior SARS-CoV-2 infection since no antibody testing was done prior to immunization. Statistical inference was constrained by the tiny sample size. Due to a longer period before sample collection, the raised antibody levels in non-lactating women following the first dosage were greater than in the lactating group.

CHAPTER VI

Conclusion and Recommendations

The placenta transmits the mother's antibodies to the fetus throughout pregnancy, and the mother's milk passes these antibodies to the infant after delivery.

Infants are protected from viral infections by the immunoglobulins in breast milk. Breast milk gives infants passive immunity and protection against Covid 19 thanks to SARS-CoV-2 antibodies discovered in moms who had a vaccination against the Covid 19 epidemic. In our study, we only looked at sIgA transfer in breast milk after Pfizer/Biontech and Coronavac vaccines. More extensive studies are needed for other vaccine types.

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Appendix A

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi :24.02.2022

Toplantı No :2022/100

Proje No :1496

Yakın Doğu Üniversitesi SHMYO öğretim üyelerinden Doç. Dr. Meryem Güvenir'in sorumlu araştırmacısı olduğu, YDU/2022/100-1496 proje numaralı ve **"COVID-19 Aşılı Emziren Kadınların Anne Sütünde SARS-COV-2 Spesifik IgA Tespiti"** başlıklı proje önerisi kurulumuzca değerlendirilmiş olup, etik olarak uygun bulunmuştur.

Prof. Dr. Şanda Çalı

Yakın Doğu Üniversitesi

Bilimsel Araştırmalar Etik Kurulu Başkanı

Kurul Üyesi	**Toplantıya Katılım**	**Karar**
	Katıldı(✓)/ Katılmadı(X)	*Onay(✓)/ Ret(X)*
Prof. Dr. Tamer Yılmaz	✓	✓
Prof. Dr. Şahan Saygı	✓	✓
Prof. Dr. Nurhan Bayraktar	✓	✓
Prof. Dr. Mehmet Özmenoğlu	*X*	*X*
Prof. Dr. İlker Etikan	✓	✓
Doç. Dr. Mehtap Tınazlı	*X*	*X*
Doç. Dr. Nilüfer Galip Çelik	✓	✓
Doç. Dr. Emil Mammadov	✓	✓
Doç. Dr. Ali Cenk Özay	✓	✓

https://etikkurul.neu.edu.tr/

Appendix X

Turnitin Similarity Report

DETECTION OF SARS-COV-2 SPESIFIC IgA ANTIBODY IN THE HUMAN MILK OF COVID-19 VACCINATED LACTATING WOMEN

ORİJİNALLİK RAPORU

% 15	% 12	% 11	%
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	www.ncbi.nlm.nih.gov İnternet Kaynağı	%2
2	www.scielo.br İnternet Kaynağı	%1
3	M. Azuma. "Fundamental mechanisms of host immune responses to infection", Journal of Periodontal Research, 10/2006 Yayın	%1
4	researchonline.lshtm.ac.uk İnternet Kaynağı	%1
5	www.researchgate.net İnternet Kaynağı	%1
6	www.mdpi.com İnternet Kaynağı	%1
7	hopkinshumanitarianhealth.org İnternet Kaynağı	<%1
8	www.livestocktrail.illinois.edu İnternet Kaynağı	<%1