وزارة التعليم العالي والبحث العلمي
جامعة ميسان
كلية التربية الاساسية

مجلة ميسان للدراسات الاكاديمية

العلوم الانسانية والاجتماعية والتطبيقية

ISSN (Paper)- 1994-697X
(Online)- 2706-722X

المجلد 22 العدد 48 السنة 2023

مجلة ميسان
للدراسات الاكاديمية

العلوم الانسانية والاجتماعية والتطبيقية

كلية التربية الاساسية - جامعة ميسان - العراق

ISSN (Paper)- 1994-697X
(Online)- 2706-722X

مجلد (22) العدد (48) كانون الاول (2023)

INTERNATIONAL STANDARD SERIAL NUMBER INTERNATIONAL CENTRE

OJS / PKP
www.misan-jas.com

IRAQI Academic Scientific Journals

doi

Crossref

ORCID

OPEN ACCESS

journal.m.academy@uomisan.edu.iq

رقم الايداع في دار الكتب والوثائق بغداد 1326 في 2009

ISSN (Paper) 1994-697X

Online 2706 -722X

https://doi.org/10.54633/2333-022-048-027

Assessment of the Lysozyme and Lactoferrin in the Saliva of Vaccinated Individuals against COVID-19

Hanadi Hafid Abdulkareem Al-Saad[1] , Ahmed Abd Burghal[2], Marwan Y. Al-Maqtoofi[3]

[1] University of Basrah/Department of biology/ College of Science
hanadi.hafiz.abdulkarim@gmail.com
[2] University of Basrah/Department of biology/ College of Science
ahmed.burghal@uobasrah.edu.iq, https://orcid.org/0000-0001-6347-9563
[3] University of Basrah/Department of biology/ College of Science
marwan.almaqtoofi@uobasrah.edu.iq, https://orcid.org/0000-0001-6126-7874

Abstract:

To understand the impact of vaccination against the Coronavirus (Covid-19) on human immunity and systems. This study aimed to determine the concentration of Lysozyme and Lactoferrin in salivary content. The results of Lactoferrin levels in the control group was 12.81, While individuals who were vaccinated 2 doses (less than six months) ago had a lactoferrin rate of 9.65.The results of Lysozyme concentration in control group unvaccinated individuals exhibits a mean concentration of 304.03, compared with those who received one vaccine dose less than 6 months exhibited a higher mean concentration was 338.01, while those vaccinated with two doses less than 6 months was 292.74.

Keywords: innate immunity, Covid-19, lactoferrin, lysozymes, Vaccines

Introduction

Different bacterial communities may be found in saliva, which can represent dietary habits and health state as well as add to the variety of food perception through sensory analysis. The similarities and contrasts between saliva in healthy conditions have not been sufficiently discussed, with many accounts of the variety of the salivary microbiome concentrating on the alterations brought about by specific disease states (Ruan *et al*., 2022), Saliva includes several anti-infective agents, including the most prevalent lysozyme, lactoferrin, and other compounds, which may help stop the invasion of oral viruses (Hayashi *et al*., 2017). One of the most important ways to combat COVID-19 is immunization, which is becoming increasingly commonplace globally. The microbiomes in body fluid of those who have received vaccinations may have changed, but these microbiomes have not yet been examined (Hosomi & Kunisawa, 2020). Saliva is one of several physiological secretions that contain peptides that act as microbial killing agents, which are immune components. These peptides have demonstrated their antiviral capabilities, and some studies indicate that they could contribute to COVID-19 protection (Brice and Diamond, 2020; Shafqat *et al.*, 2022; Huan *et al.*, 2020). By hydrolyzing the β-glycosidic link between N-acetylglucosamine and N-acetylmuramic in the

bacterial cell wall by lysozyme, leading to the efficient elimination of them, especially Gram-positive bacteria (Ragland and Criss, 2017).

However, its antibacterial properties go beyond enzymatic activity as the cationic properties of lysozyme allow it to adhere to negatively charged surfaces, similar to lactoferrin iron-binding glycoprotein of the transferrin family, One of the elements of the body's immune system, which is primarily found in mucus and has antibacterial action (Bactericide and Fungicide) (Sanchez *et al*., 1992; Ragland and Criss, 2017;Woods *et al*., 2011). LF has bacteriostatic effects against a variation of Gram-positive and Gram-negative bacteria. inhibits bacterial adhesion to the host due to its high affinity to iron (Wang *et al*., 2019). In addition to the antibacterial activity, many studies have demonstrated that LF also exhibits antiviral activity on both DNA- and RNA-viruses. This antiviral effect is proven to be achieved by LF's ability to block cellular receptors or binding to the virus particles (Zhang *et al*., 2021). The aim of this study was to assess the levels of antimicrobial peptide lysozyme and lactoferrin, in the saliva of Iraqi individuals who received the COVID-19 vaccine. By examining these factors, to gain a better understanding of how COVID-19 vaccination affected the immune response of the Iraqi population.

Material and methods

Sample collection

A total of 80 samples were gathered from individuals who assessed health care centers in Basra Governorate for the period from Sept. 21, 2022 to Jan. 21, 2023. The saliva samples were collected in a sterile container from individuals who had contracted COVID-19 in the past but had not received a vaccination. These individuals did not have any immune suppressors, making them the control group for this study. On the other hand, the study group consisted of patients who had been infected with COVID-19 and had received vaccinations. They also did not have any immune suppressors. The saliva samples collected from the individuals were transferred directly to the laboratory to be preserved at 4°C. Lactoferrin and lysozyme analysis were performed at Al-Qurna General Hospital.

Immunotherapy Assay:

To obtain transparency, saliva samples were centrifuged for 20 min. at 1000 rpm. ELISA method was used to measure a concentration of Lactoferrin, and Lysozyme in each sample. The results obtained by the ELISA technique in saliva samples of individuals who received the COVID-19 immunization and those who did not were compared for lactoferrin and lysozyme levels (figure 1). Testing human lysozyme and Lactoferrin usage for immunotherapy as a natural immune system production was provided by (AL-shkairate establishment for medical supply. Amman, Jordon) as a powder processed through dilution and aliquot for preparation final concentration 20ng/ml, 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.313ng/ml and blank zero under manufacture instructions

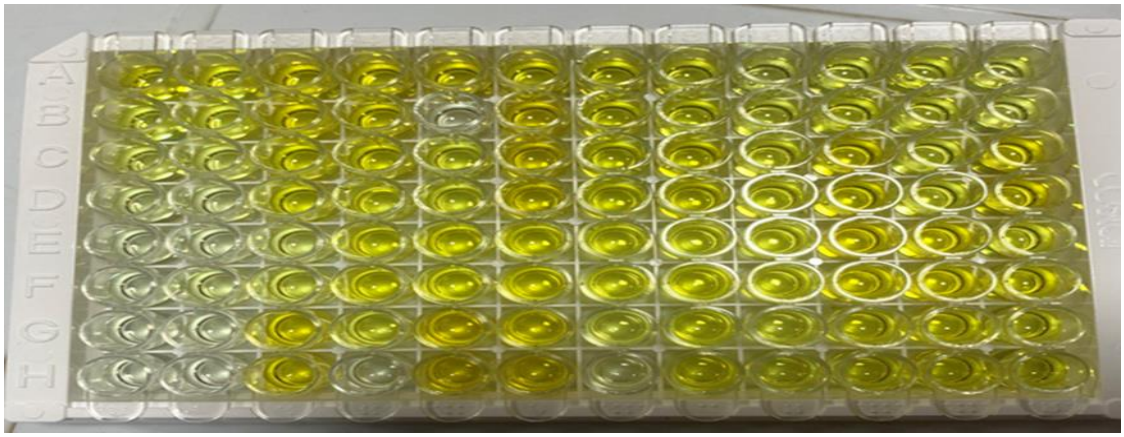


Figure 1: ELISA micro-titer panel to detect the levels of lactoferrin and lysozyme in

ISSN (Paper)-1994-697X (Online)-2706-722X

different groups of vaccinated and unvaccinated individuals..

Results and discussion

Based on gender, the results showed that female (68%) more than male (31%) (Figure 2).

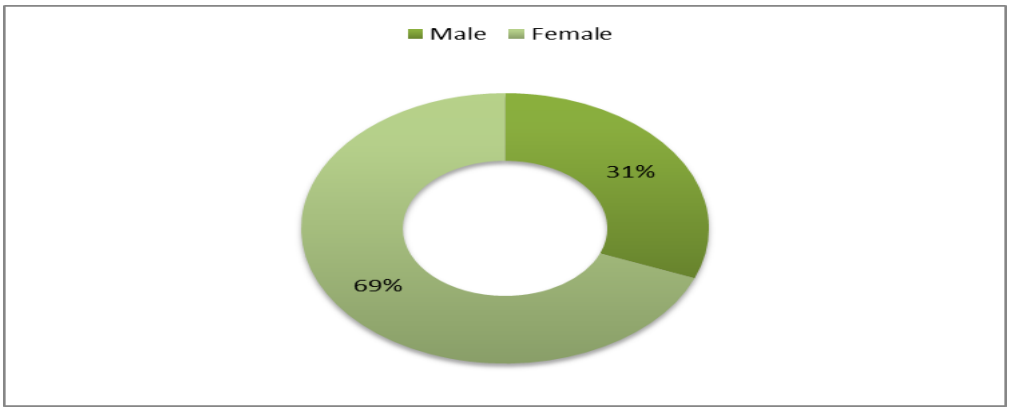


Figure 2: Percentage of individuals participating in the current study and their distribution by gender

In Figure 3, the results demonstrate that the highest percentage 45.0% of participants belonging to age group (<20 year), while the lowest percentage 5.0% of the study samples belonging to age group (≥ 50 years).

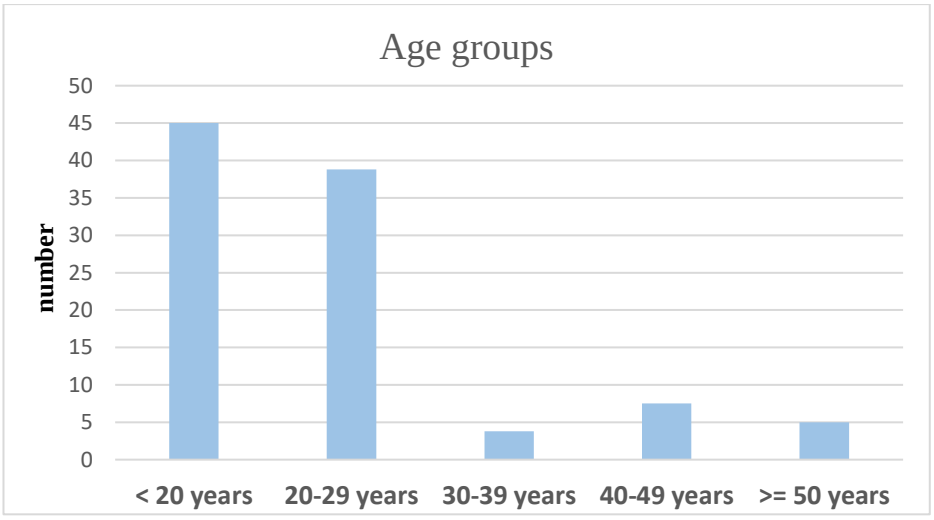


Figure 3: Vaccinated people in the current study to determine immune parameters by age group.

Table 1 presents a comparison of the levels of lactoferrin in a (G1) Control group and Vaccinated individuals under different dosage conditions. The Control group's mean lactoferrin level in the first comparison is 12.8 with an SD of 8.4. The mean Lactoferrin level in the Vaccinated People group, which received both doses at least six months ago, is 9.6, with a standard deviation of 5.8. There may not be a statistically significant difference in the amounts of lactoferrin among these 2 groups, referring to the stated (P>0.05). In the second comparison, those who had their vaccinations less than six months ago are compared to the Control group. The vaccinated group shows an increase in lactoferrin concentration of 15.3, compared to the control group, which retains the same mean lactoferrin level of 12.8. The stated P-value of 0.44. The groups of control and vaccinated who got two doses through six months ago are compared in the third comparison. While the Vaccinated People group has a mean lactoferrin level of 14.0, the Control group retains a mean lactoferrin level of 12.8. There appears to be no significant (P>0.05) in the amounts of lactoferrin between these 2 groups, as indicated by the reported p-value of 0.63. The results showed, that there is no proof of significant variations in levels of lactoferrin among the two groups (control and vaccinated), according to the supplied p-values as showed in table 1.

ISSN (Paper)-1994-697X (Online)-2706-722X

Table 1: Results of lactoferrin concentration among different vaccination groups compared with the control group

	(G)Groups	NO.	Mean	S.D.	S.E.	P. value
Lactoferrin	Control G1	24	12.8	8.4	1.7	0.15
	Vaccinated People G2 (2 doses more 6 months)	21	9.6	5.8	1.2	
	Control G1	24	12.8	8.4	1.7	0.44
	Vaccinated People G3 (1 dose less 6 months)	18	15.3	12.7	2.9	
	Control G1	24	12.8	8.4	1.7	0.63
	Vaccinated People G4 (2 dose less 6 months)	17	14.0	8.1	1.9	

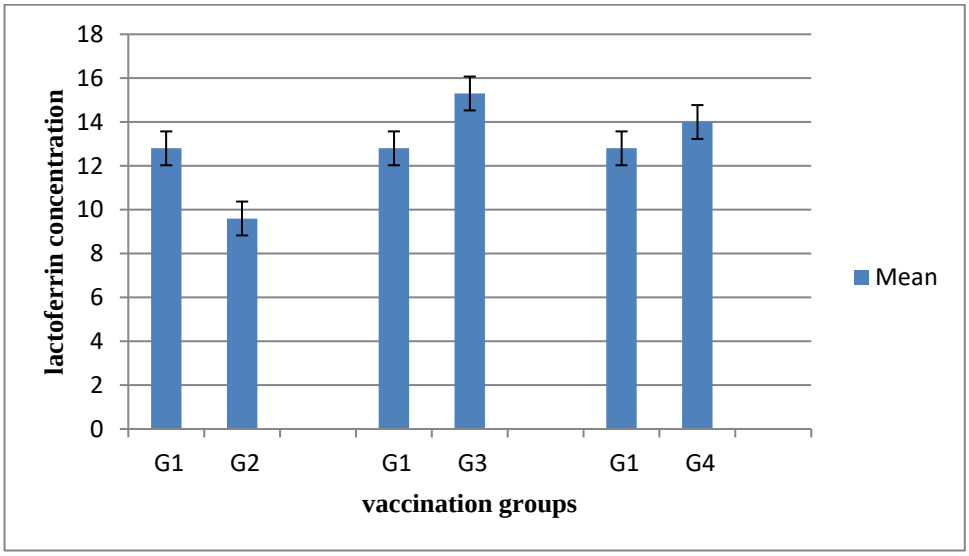


Figure 4: Groups of individuals according to vaccine doses to determine lactoferrin concentrations in saliva samples: G1 (Control group), G2 second group (Vaccinated two doses over 6 months), G3 third group (Vaccinated by a single dose of less than six months), and G4, fourth group, were (Vaccinated with two doses within six months).

Lactoferrin concentration in saliva samples from COVID-19 vaccination recipients and non-recipients. Based on the number and duration of the vaccination, the samples were split into three groups, plus an extra unvaccinated control group. A microtiter plate was used to quantify lactoferrin, and the findings were compared to typical reference ranges. Although there were variations in the concentrations between the groups, there were no discernible changes from the control group in terms of statistics. These results provide insight into the differences in lactoferrin levels between groups.

Analysis of lactoferrin levels in the studied samples can shed light on the immune system's reaction to the vaccine. Saliva and other body fluids contain lactoferrin, which is essential for innate immunity. After vaccination, differences in lactoferrin levels may indicate the initiation of innate immune responses in mucosal cells, which can determine whether or not infection with the virus occurs (Einerhand *et al*., 2022). Furthermore, it has been demonstrated that lactoferrin exhibits antiviral action against a range of viruses, including some coronaviruses. As a result, monitoring salivary lactoferrin levels may be also reveal details about the vaccine's possible ability to protect against that virus. Lactoferrin levels were tested in saliva samples from symptomatic and asymptomatic COVID-19 patients, as well as healthy controls, in a study by De Figueiredo *et al*. (2021). Although lactoferrin concentrations differed between the two groups, COVID-19 patients and controls were not statistically significantly different, according to study results.

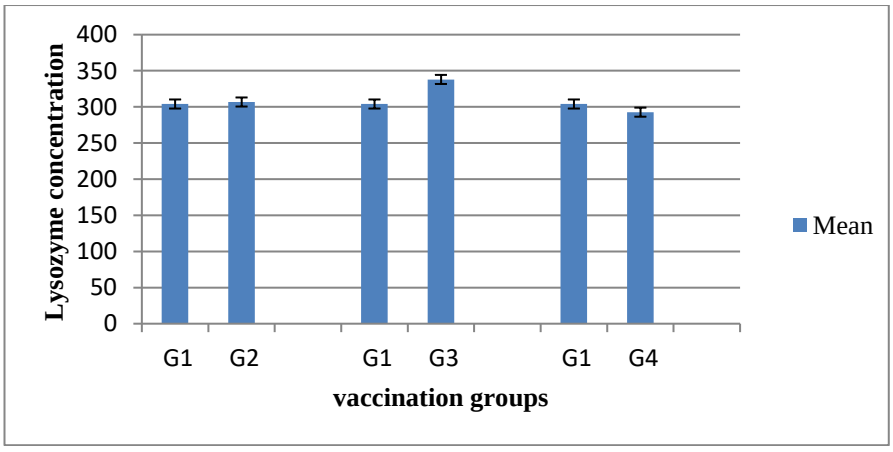
ISSN (Paper)-1994-697X (Online)-2706-722X

This conclusion is in line with the current study, which showed that there were no appreciable variations in lactoferrin concentrations among the control and vaccinated groups. In general, quantifying lactoferrin in saliva samples from COVID-19 vaccine recipients might help us better understand the immunological response to vaccination and how it may affect the effectiveness of vaccines, while specific research on lactoferrin's role in vaccinated individuals is limited, its immunomodulatory properties may contribute to a more balanced immune response following vaccination. By promoting a robust but controlled immune response, lactoferrin may enhance vaccine efficacy and help mitigate potential adverse reactions (Yamamoto, 2022).

The table 2 presents information on Lysozyme concentration in saliva samples from different groups, including unvaccinated individuals and those vaccinated with varying dosing intervals. The Control group of unvaccinated individuals (N=24) exhibits a mean Lysozyme concentration of 304.03, with a standard deviation of 184.71 and a standard error of 37.70. The associated p-value of 0.95 indicates no statistically significant difference in Lysozyme concentration. Similar findings are observed in the comparison with vaccinated individuals who have received two doses more than 6 months ago, where the mean concentration is 306.78, but without a provided p-value. Notably, The unvaccinated people group again corresponded with the vaccinated group by one dose less than 6 months (N=18) and exhibited a higher mean concentration of 338.01, although the missing p-value prevents assessing statistical significance. In addition, the Control group is compared twice more: one with those vaccinated with two doses less than 6 months ago (N=17, mean=292.73) and another without a provided p-value. Ultimately, the analyses indicate that there are very minor statistically significant variations in Lysozyme concentrations between the vaccinated with different doses and the unvaccinated group.

Table 2: Lysozyme levels in saliva individuals of vaccinated and Unvaccinated virus corona Vaccine.

	Groups	NO	Mean	SD	SE	P. value
Lysozyme	Control G1	24	304.03	184.71	37.70	0.95
	Vaccinated People G2 (2 doses more than 6 months)	21	306.78	119.51	26.07	
	Control G1	24	304.03	184.71	37.70	0.48
	Vaccinated People G3 (One dose less 6 months)	18	338.01	105.79	24.93	
	Control G1	24	304.03	184.71	37.70	0.82
	Vaccinated People G4 (2 dose less 6 months)	17	292.73	103.08	25.00	



ISSN (Paper)-1994-697X (Online)-2706-722X

Figure 5: Groups of individuals according to vaccine doses to determine lysozyme concentrations in saliva samples: G1 (Control group), G2 second group (Vaccinated two doses over 6 months), G3 third group (Vaccinated by a single dose of less than six months), and G4, fourth group, were (Vaccinated with two doses within six months).

The results showed the concentration of lysozyme in samples of individuals under the current study for various groups that included vaccinated and unvaccinated individuals. Lysozyme levels were measured using an ELISA panel, and the results were compared to the control group. From the results, it was found that there were no statistically significant differences (P value: 0.95, 0.48 and 0.82 respectively) between the groups that included those vaccinated with different doses and those not vaccinated with the Coronavirus vaccine, as shown in Table 2 and Figure 5. These results provide light on the differences in lysozyme concentration between groups of those who received the Coronavirus immunization and those who did not. Since saliva is a typical sample of the mucosal environment, measuring the amounts of lysozyme in saliva samples can help to better understand how the vaccination affects mucosal immune responses. Variations in lysozyme levels may be a sign of modifications to the innate immune defense system, which might indicate how well the vaccination stimulated an immunological response. Furthermore, lysozyme levels between vaccinated and unvaccinated controls may be compared to learn more about how the vaccine affects the immune system and if it can help to improve mucosal immunity (Qaysar *et al*., 2022).

It is important to know that in order to determine the true effect of vaccination on the immune system, there is a need to further study the correlation between clinical results and lysozyme values in relation to vaccination against the Coronavirus. Determining lysozyme is one way to evaluate the immune response and determine how well vaccination protects against virus infection.

Conclusion

The results of the study indicate the importance of measuring the levels of immune parameters such as (lactoferrin, lysozyme) and others in samples of immunized and non-immunized individuals. ELISA technology provides qualitative results to evaluate the immunogenicity associated with the efficacy of vaccines.The results showed that there was no significant difference in the concentrations of the immunological markers used in this study between the different groups compared to the control samples. It is worth noting that more studies must be done to understand the immune response and its relationship to vaccination.

Acknowledgements

The authors thank the University of Basra, College of Science, Department of Biology, the staff of Al-Qurna General Hospital, and all the individuals involved in this study.

References

Brice, D. C., & Diamond, G. (2020). Antiviral activities of human host defense peptides. *Current Medicinal Chemistry*, *27*(9), pp.1420–1443. https://DOI:10.2174/0929867326666190805151654

De Figueiredo, C. S., Sandre, P. C., Portugal, L. C. L., Mázala-de-Oliveira, T., da Silva Chagas, L., Raony, Í., Ferreira, E. S., Giestal-de-Araujo, E., Dos Santos, A. A., & Bomfim, P. O.-S. (2021). COVID-19 pandemic impact on children and adolescents' mental health: Biological, environmental, and social factors. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *106*, 110171. https://doi.org/10.1016/j.pnpbp.2020.110171.

Einerhand, A. W. C., van Loo-Bouwman, C. A., Weiss, G. A., Wang, C., Ba, G., Fan, Q., He, B., & Smit, G. (2022). Can Lactoferrin, a Natural Mammalian Milk Protein, Assist in the Battle against COVID-19? *Nutrients*, *14*(24), 5274. https://doi.org/10.3390/nu14245274.

ISSN (Paper)-1994-697X (Online)-2706-722X

Hayashi, T., To, M., Saruta, J., Sato, C., Yamamoto, Y., Kondo, Y., Shimizu, T., Kamata, Y., & Tsukinoki, K. (2017). Salivary lactoferrin is transferred into the brain via the sublingual route. *Bioscience, Biotechnology, and Biochemistry*, *81*(7), pp.1300–1304. https://doi.org/10.1080/09168451.2017.1308241.

Hosomi, K., & Kunisawa, J. (2020). Impact of the intestinal environment on the immune responses to vaccination. *Vaccine*, *38*(44), pp.6959–6965. https://doi.org/10.1016/j.vaccine.2020.08.079.

Huan, Y., Kong, Q., Mou, H., & Yi, H. (2020). Antimicrobial peptides: classification, design, application and research progress in multiple fields. *Frontiers in Microbiology*, 2559. https://doi.org/10.3389/fmicb.2020.582779

Qaysar Musa, S., Mohammed AliJassim, M., & Mohammed Mahmood, M. (2022). Tracing Some Salivary Immune Elements in Iraqi SARS-2 Patients. Archives of Razi Institute, 77(5), pp.1587-1591. https://doi.org/10.22092/ari.2022.358330.2195

Ragland, S. A., & Criss, A. K. (2017). From bacterial killing to immune modulation: Recent insights into the functions of lysozyme. *PLoS Pathogens*, *13*(9), e1006512. https://doi.org/10.1371/journal.ppat.1006512.

Ruan, X., Luo, J., Zhang, P., & Howell, K. (2022). The salivary microbiome shows a high prevalence of core bacterial members yet variability across human populations. *Npj Biofilms and Microbiomes*, *8*(1), 85. https://doi.org/10.1038/s41522-022-00343-7

Sanchez, L., Calvo, M., & Brock, J. H. (1992). Biological role of lactoferrin. *Archives of Disease in Childhood*, *67*(5), 657.

Shafqat, F., Rehman, S. U., & Niaz, K. (2022). Lactoferrin can attenuate SARS-CoV-2: an analysis of evidential relations. *Biomedical Research and Therapy*, *9*(2), pp.4901–4919. https://doi.org/10.15419/bmrat.v9i2.727

Wang, B., Timilsena, Y. P., Blanch, E., & Adhikari, B. (2019). Lactoferrin: Structure, function, denaturation and digestion. *Critical Reviews in Food Science and Nutrition*, *59*(4), pp.580–596. https://doi.org/10.1080/10408398.2017.1381583

Woods, C. M., Hooper, D. N., Ooi, E. H., Tan, L.-W., & Carney, A. S. (2011). Human lysozyme has fungicidal activity against nasal fungi. *American Journal of Rhinology & Allergy*, *25*(4), pp.236–240. https://doi.org/10.2500/ajra.2011.25.363

Yamamoto, K. (2022). Adverse effects of COVID-19 vaccines and measures to prevent them. *Virology Journal*, *19*(1), pp.1–3. https://doi.org/10.1186/s12985-022-01831-0

Zhang, Y., Lu, C., & Zhang, J. (2021). Lactoferrin and its detection methods: A review. *Nutrients*, *13*(8). https://doi.org/10.3390/nu13082492