Research Article

Cellular and humoral immunity assessment in recovered COVID-19 Iraqi dentists

Ferial M. Abdulrida[1*], Ameena R. Diajil[1], Michaela L. Goodson[2]

1 Department of Oral Diagnosis, College of Dentistry, University of Baghdad, Baghdad, Iraq.
2 Newcastle upon Tyne, Newcastle University, England, United Kingdom
* Corresponding author: drfmahmoodabdulrida@codental.uobaghdad.edu.iq

Received date: 10-09-2023
Accepted date:28-10-2023
Published date:15-03-2025

Article DOI

Abstract: Background: Dentists, safeguarding against contagious diseases, face infection risks. The study aimed to assess post-COVID immunity, quantification of salivary biomarkers for prognostication, and immune surveillance. Materials and methods: A cross-sectional study was done on 91 working Iraqi dentists from June to August 2022. The dual IgG s1 &n, and IgA s1 &n specific to COVID-19 were measured by ELISA-specific kits from serum and saliva. From randomly selected 36 out of 91 participants CD4 and subtypes TH1 and TH2 were counted by flow cytometry from fresh whole peripheral blood. Results: All CD4, Th1, and Th2 percentage levels were reduced as a whole if compared to known normal value, and Th2 elevated and inhibited the level of Th1 in all study individuals. All cells were significantly associated with a positive history of COVID-19 infection whereas the CD4 was significantly related to the Pfizer type of vaccine, loss of both sense and recovery time within 15 days. A positive correlation was found between CD4 and Th2 and CD4 with IgG n in serum; this antibody was highly significant with positive COVID-19 infection higher than that of serum IgG s1. Noticeably, the IgA (s+n) in serum was associated with a positive history of infection and could be detected in individuals with a duration of the last infection >1-2 years and last vaccine duration > 6- 12 months**.** Conclusion: A low percentage level of CD4 and an imbalance Th1/Th2 ratio made the recovered individual more susceptible to re-infection but the significantly high percentage of specific COVID antibodies followed one time of infection or booster vaccine dose gave their protection.

Keywords: Post covid-19, CD4, Th1 and Th2, IgG, IgA.

Introduction

Antibodies are proteins synthesized after infection or vaccination against an infection. They can protect from infection, or severe illness after infection, but the period they last differs for each person and depends on the disease pathogenesis. An antibody test may not show current infection, because antibodies are only detected 1 to 3 weeks post-infection [(1)]. Antibody levels; may correlate with the clinical severity of the disease [(2)]. Mild and asymptomatic infections result in weaker immune response [(3)].In COVID-19 patients, nucleocapsid detection demonstrates that viral RNA is securely stored and protected from the host environment aiding replication and propagation of the virus [(4)].

Anti-N and anti-S (IgG) antibodies, along with other proteins like Spike (S1), Receptor Binding Domain (RBD), membrane, and envelope, are significant because they have been found to remain in the body for extended periods [(5)]. Measuring both IgA and IgG antibodies together offers a dependable method for assessing COVID-19 incidence, especially in populations with low antibody prevalence [(6).] The anti-RBD IgA antibodies are short-lived and persist for up to 2 months after the onset of symptoms (5). IgA antibody determination used in identifying an acute/recent SARS-CoV-2 infection [(5)].

The Nucleocapsid (N) protein is a target protein for vaccine development (6) The (N) protein acts as a representative protein for SARS-specific T-cell proliferation in a vaccine setting [(7)]. The IgG, IgA, and IgM antibodies acting against (N) proteins can be identified in COVID-19 patients [(4)]. Antibodies targeting the S1 protein have a greater neutralizing capacity than those directed at the N protein, owing

to their role in infection [8]. Detecting antibodies to the spike (S1 and RBD) proteins of SARS-CoV-2 is crucial for understanding a patient's immune response and identifying N antibodies [9]. In contrast to anti-S1 and anti-RBD antibodies, anti-N antibodies typically do not have neutralizing effects, and no direct link has been found between the levels of anti-N antibodies and neutralizing antibody titers, unlike the relationship observed with anti-S1 and anti-RBD antibodies. It's also important to note that not all anti-S1 and anti-RBD antibodies are capable of neutralizing. [10]. Antibody testing does not assess an individual's protection against severe COVID-19 infection or demonstrate the need for vaccination in an unvaccinated person [11].

SARS-CoV-2-specific antibody screening plays a crucial role in evaluating the effectiveness of vaccines (12). It helps quantify how quickly antibodies develop after vaccination to combat various SARS-CoV-2 strains [13]. A third dose (booster) of the vaccine can stimulate stronger antibody responses [14, 15]. Clinically meaningful levels of anti-spike IgG antibodies may be seen between the second and third doses, with an increase ranging from 5% to 45% [16]. Several factors influence this increase, such as whether the same vaccine is used for all three doses or if a different vaccine is used for the booster (heterologous vaccination) [17]. When interpreting immunity results, it is important to consider both the timing between the second and third doses and the timing of serum collection after the third dose. A heterologous vaccination regimen may offer enhanced serological protection for patients. For example, primary vaccination with AstraZeneca (OxA) has been associated with higher neutralizing antibody responses against both the Beta and Delta variants of SARS-CoV-2 [18].

In terms of booster vaccinations, studies have shown that homologous regimens, such as Moderna (MDN) and OxA, result in higher antibody responses compared to Pfizer-BioNTech (BNT) vaccinations [19]. MDN-vaccinated individuals have demonstrated a stronger serological response against Omicron than BNT-vaccinated individuals after two doses [20]. However, patients who received the BNT booster exhibited a higher serological response compared to those who only received the MDN vaccine [21].

Assessing antibodies to both the S and N proteins enhances the sensitivity and reliability of detecting antibodies against the virus. Additionally, understanding the varying IgG responses of N and S antibodies, as well as their long-term kinetics, is vital for effectively characterizing patients both during SARS-CoV-2 infection and after vaccination [17].

A detectable T-cell response after the second dose of the vaccine, which further increases following the booster dose, suggests that the third vaccination enhances anti-SARS-CoV-2 T-cell immunity [22]. For individuals with a limited serological response after two doses, the booster dose can help stimulate anti-SARS-CoV-2 T-cell activity [19]. T-cell counts may also be slightly elevated in those who received the booster compared to those who did not [21]. CD4+ helper T cells are a heterogeneous group crucial for regulating immune responses and are involved in various processes, including infection, autoimmune diseases, cancer progression, and chronic inflammation [23]. These cells are classified into subgroups based on distinct transcription factors and cytokine profiles: Th1 cells, which express T-bet, produce Tumor Necrosis Factor-alpha (TNF-α), Interferon-gamma (IFN-γ), and Interleukin-2 (IL-2); and Th2 cells, characterized by the expression of GATA-3, which secrete IL-4, IL-5, and IL-13 (24). Research on SARS-CoV-1 infections has shown that while antibody levels typically decline within 1–2 years post-infection, T-cell responses can last for up to 17 years [25].

Peripheral T-cell depletion is closely associated with adults who have COVID-19, with the degree of depletion being positively correlated with disease severity. In contrast, asymptomatic individuals and children typically maintain higher peripheral T-cell counts (26). While pre-existing cross-reactive T cells may help accelerate the clearance of SARS-CoV-2, their exact role remains uncertain. However, there is evidence suggesting that a well-balanced T-cell response could help prevent or reduce the severity of COVID-19, while a delayed or insufficient response may contribute to increased tissue damage [27].

The presence of a high number of Th1 lymphocytes and inflammatory monocytes in bronchoalveolar lavage and lung biopsies from critically ill COVID-19 patients suggests that an excessive cellular immune response may severely impact lung function by disrupting the pulmonary microcirculation. This cellular immune response to the viral infection is primarily driven by interferon (IFN), with type I IFN playing a crucial role in this process [28].

The ratio of Th1 to Th2 responses plays a significant role in determining the outcome of COVID-19. A well-regulated Th1 immune response is essential for effectively clearing the virus once it is detected. However, if this response becomes dysregulated, it can lead to an exaggerated immune reaction, triggering a cytokine storm that activates Th2 cells and diminishes the number of Th1 cells, thereby increasing the presence of activated Th2 cells [29]. In COVID-19 patients, an excessively active Th2 response has been linked to worse prognoses, with Th2 cells being identified as an independent risk factor for mortality, especially in patients with high total lymphocyte counts [30]. This imbalance may result in excessive activation and proliferation of various immune cells, including neutrophils, macrophages, dendritic cells, mast cells, and Th17 cells, fueling uncontrolled inflammation driven by innate immune cells and potentially leading to tissue damage [31].

Methods

This cross-sectional study was conducted on 91 dentists from Baghdad City working in primary health centers and private clinics. Following ethical approval (Ethics Committee for Research from the College of Dentistry, University of Baghdad, Baghdad, Iraq, under protocol number (460722) and informed consent, Sample collection was undertaken between 9th March 2022 to 21st September 2022; Participants vaccinated with vaccines other than Pfizer or AstraZeneca were excluded from the study.

A questionnaire was completed by one examiner which included demographic information, medical history, COVID-19 infection data, and others.

Sample collection

Each participant underwent saliva, and blood sample collection by the same examiner. The whole saliva was centrifuged and the supernatant was collected in a microcentrifuge tube. Blood serum was collected in a microcentrifuge tube and stored at -4C. Whole fresh blood was collected daily from participants using an Ethylenediaminetetraacetic acid (EDTA) anticoagulant tube. Around 1ml was prepared for detection of CD4, Th1, and Th2 by flow cytometry, procedure, and kit from "Biolegend enabling legendary discovery", United States.

Following collection and storage, serum and saliva samples were applied to a sterile 96-well plate for ELISA analysis to detect the Antibodies.

The ELISA kit utilized indirect enzyme-linked immunosorbent assay (ELISA) technology. SARS-CoV-2 antigens (N and S proteins) were pre-coated onto 96-well plates. Test samples were added to the wells, and unbound conjugates were removed by washing with a wash buffer. Biotin-conjugated anti-human antibodies were introduced, followed by another wash with the buffer. Next, Horseradish peroxidase (HRP)-streptavidin was added, and unbound conjugates were washed away. Tetramethylbenzidine (TMB) substrates were employed to visualize the HRP enzymatic reaction, where TMB catalyzed by HRP produced a blue color that turned yellow after the addition of an acidic stop solution. The optical density of the resulting color was measured using a 'Huma Reader HS device' at 450 nm, with a reference wavelength set to 650 nm. The following kits were used in this study: Human Anti-2019 nCoV IgA ELISA Kit V2.1 Catalog No.: MBS7612290 x2; Human anti-SARS-CoV2(N) IgG ELISA Kit V1.5 Catalog No.: MBS7608188 x2; Human SARS-CoV-2 Spike Protein S1IgG manual version:18.12.1 Catalog No.: MBS2614310 x2. All six kits are designed for the quantitative detection of anti-SARS-CoV-2 antibodies in serum, plasma, and other biological fluids.

Statistical analyses

The detailed description of each variable was conducted using the Statistical Package for Social Sciences (SPSS) version 26. Participant details were linked to serial numbers, and the collected data was managed daily. Data were presented as mean ± standard deviation or frequency/percentage, depending on the variable type. To assess differences between two independent continuous variables, an independent sample t-test (for normally distributed data) and Mann-Whitney U test (for non-normally distributed data) were used. For comparisons involving more than two independent continuous variables, the ANOVA test (for normally distributed data) and Kruskal-Wallis test (for non-normally distributed data) were applied. The chi-square test and Fisher's exact test were used to assess the relationship between categorical variables. Correlation tests were used to assess the strength and direction of the relationship between the studied variables. A confidence level of 95% with a P-value equal to or less than 0.05 was considered significant.

Results

Based on the initial demographic and COVID history questionnaire taken during the first examination, the 91 participants were classified into four groups. 19 were "Asymptomatic and had not been diagnosed with covid", 26 were classed as previously "Infected", as they had a history of infection or not; 25 as "Booster", 21 as "Unvaccinated" according to the number of doses of vaccine taken Table 1.

Table 1: Demographic features of the participants.

Variables	Asymptomatic		Infected		Booster		unvaccinated	
	Frequency No.=19	%	Frequency No.=26	%	Frequency No.=25	%	Frequency No.=21	%
Age groups								
≤35 years	15	78.9	19	73.1	18	72	16	76.2
>35 years	4	21.1	7	26.9	7	28	5	23.8
Gender								
Male	6	31.6	9	34.6	8	32	10	47.6
Female	13	68.4	17	65.4	17	68	11	52.4

Humoral immunity

The serological antibodies used in this study were IgG and IgA for both serum samples and saliva samples, the IgG and IgA were specific for both spike protein and nucleocapsid protein of the COVID-19 virus. The result of these ELISA kits for those specific proteins was calculated quantitively and qualitatively. The' Huma reader HS device' was used on wavelength 450nm for reading and calculation from point to point.

Regarding the IgA level in serum the highest value in the infected group (5.1±4.9) followed by the booster group (4.8±4.1), the unvaccinated group had a higher level of IgA in serum than saliva (3.2±4.1) while the lowest serum level of IgA showed in the asymptomatic group (2.2±2.4) which was the only group had saliva IgA level higher than that in serum. The quality of IgA in saliva and serum also showed a variation within the groups of this study. Unlike the quantity value of IgA in serum, the positive IgA (qualitative) level in serum was higher than in saliva in all groups. The highest positive level of IgA in serum was expressed in the infected group (23;92.3%) and the lowest level in the

asymptomatic group (11;57.9%). The highest positive level of saliva IgA was in the booster group (15;60%) and the lowest level of it was in the unvaccinated group (8;38.1%), Table 2.

Table 2: The quantities and qualitative values of Antibodies (ng/ml) related to COVID-19 participants:

Antibodies	Study groups			
	Asymptomatic	Infected	Booster	Unvaccinated
Quantitively mean ±SD (ng/ml)				
Saliva IgA	3.8±3.8	3.8± 4.3	3.5± 3.6	2.7± 2.8
Serum IgA	2.2±2.4	5.1± 4.9	4.8 ± 4.1	3.2± 4.1
Saliva IgG s1	30.9±10.8	36.2±10.6	36.9±12.4	29.1±14.1
Serum IgGs1	36.8±23.7	45.2±27.9	49.6±27.8	37.1± 28.2
Saliva IgG n	28.0±16.9	23.1±16.5	27.4±14.2	18.7±10.9
Serum IgG n	36.1±23.5	40.3±15.9	44.1±18.6	30.9±17.1
Qualitative Percentage %				
Saliva IgA	42.1	50	60	38.1
Serum IgA	57.9	92.3	92	66.7
Saliva IgG s1	47.4	76.9	80	47.6
Serum IgGs1	47.4	69.2	76	38.1
Saliva IgG n	47.4	38.5	64	33.3
Serum IgG n	63.2	92.3	92	66.7

The Relation of IgA Serum with Other Variables

The quality level of serum IgA was significantly related to the presence of infection (Person Chi-square=33.37; p-value =0.000) or (Fisher's Exact=17.18; p value=0.001 51/91 participants showed the presence of IgA in serum with a history of infection compared to 18 with positive IgA level but with no infection history.

A significant relationship could be found between the serum level of IgA and the duration of the last infection (Person Chi square=15,97; p-value 0.043) or (Fisher's Exact=16.13; p-value=0.016). A high proportion of participants with serum IgA positive value was shown in whom had a duration of last infection from 1-2 years (22; >1-2years); more than that with last infection duration 6-12months (17;>6months-1year) and the lowest proportion of individuals with serum IgA positive and had last infection duration less than 6months (14; ≤ 6months). Regarding the last vaccination dose with serum quality of IgA, a significant relationship was found between the positive value of IgA and the duration of the last vaccine dose (Person Chi square=13.53; p-value=0.035) or (Fisher Exact=11.75; p-value=0.033). The highest value of IgA was expressed in the last duration of the vaccine (35;>6 months) followed by (23; < 6 months), Figure 1(A, B).

The IgG Spike1 protein in Saliva and Serum

The mean value of IgG to S1protien in serum and saliva showed the same level of elevation in all study groups with a higher level in serum than in saliva, the highest level of these antibodies showed in the booster group of both serum and saliva (49.6±27.8; 36.9±12.4) respectively whereas the lowest value in serum showed in the asymptomatic group (36.8±23.7) and that of saliva in the unvaccinated group (29.1±14.1), Table 2.

Whereas the quality level of IgG to S1protien showed a variation from the quantity value within each study group regarding saliva or serum, the highest positive value of this antibody in saliva showed in the booster group similar to the quantity value (20;80%) while the lowest positive value showed in the

asymptomatic group (9;47.4%). The serum's positive quality value also showed the highest value within the booster group (19;76%) while the lowest positive value was in the unvaccinated group (8;38.1%), Table 2.

The Relation of IgG S1 with other variables

The serum IgG S1 percentage value was significantly related to olfactory clinical dysfunction (Person Chi square=23.6; p-value=0.024) or (Fisher's Exact=27.9, p-value=0.006). As more positive values expressed moderate and mild scores of hyposomnia, severe and complete anosmia showed in low IgG s1 values. The saliva IgG s1 value percentage was significantly related to loss of sense and a high percentage of IgG S1 in saliva expressed in the loss of both senses, figure (1. E, F). The serum IgG S1 value percentage was significantly related to times of infection (Fisher's Exact test=11; p-value=0.05) as more of a positive value of serum antibodies was presented in participants with one time of infection; also was significantly related to the last vaccine dose (Person Chi-square=14.8, p-value=0.021) or (Fisher's Exact test= 13; p-value=0.022) as a high percentage value of serum IgG s1expressed in participants with last dose vaccine duration of >6 months -1year, followed by < 6months, Figure (1. D, G).

Saliva and serum IgG nucleocapsid

A specific antibody for nucleocapsid protein of the COVID-19 virus was measured in both serum and saliva, the quantity amount mean of IgG n in serum was higher than that in saliva in all study groups, and the highest value of serum IgG n mean value was showed in the booster group (44.1±18.6) while the lowest mean value of this serum antibody was in the unvaccinated group (30.9±17.1). On the other hand, the mean value of this antibody in saliva showed the highest mean value in the asymptomatic group (28.0±16.9) and the lowest mean value in the unvaccinated group (18.7±10.9). The quality value percentage of IgG n in serum was higher than that of saliva in all study groups, the highest percentage value was in the infected group (24; 92.3%) while the lowest value was in the asymptomatic group (12;63.2%), while the saliva IgG n percentage value was showed the highest percentage in the booster group (16;64%) while the lowest percentage was in the unvaccinated group (7;33.3%), Table 2.

The Relation with Other Variables

The percentage value of IgG n and IgG S1 of both serum and saliva in this study was associated with the infection status of participants (Fisher's Exact =9.39;p-value= 0.038) for serum IgG n value; and (Person Chi-square test; p-value=0.000) or (Fisher's Exact=10.6;p-value=0.018)for saliva IgG n value, as a high positive percentage of IgG n was expressed in those participants who had a positive infection more than those with negative infection history regarding both serum and saliva and (Fisher's Exact test=9.6; p-value =0.031) for salivary IgG S1and (Person Chil square=28; p-value=0.000) or (Fisher's Exact test=13; p-value=0.004) for serum IgG S1, the more positive value of antibodies participants had a history of COVID-19 infection, Figure (1-H).

Cellular immunity

Fresh whole blood in the anticoagulant tubes was randomly selected, from 36 out of 91 participants from the study groups, after being processed and read by flow cytometry the data was analyzed by FCS EXPRESS 7programme, and the following output results could be detected, Figure (2).

The average value of each three cells in the four study groups showed approximate linearity in all average values of TH1 in the four groups with an average percentage value of this cell in the booster group (2.769%) and the lowest average percentage value in the unvaccinated (1.382%). The average percentage of TH2 was higher in the asymptomatic group (10.4%) and the lowest in the infected group (7.41%). The average CD4 cell percentage was high in the unvaccinated group (22.236%) and lowest in the infected group (15.756%), Figure (3-A).

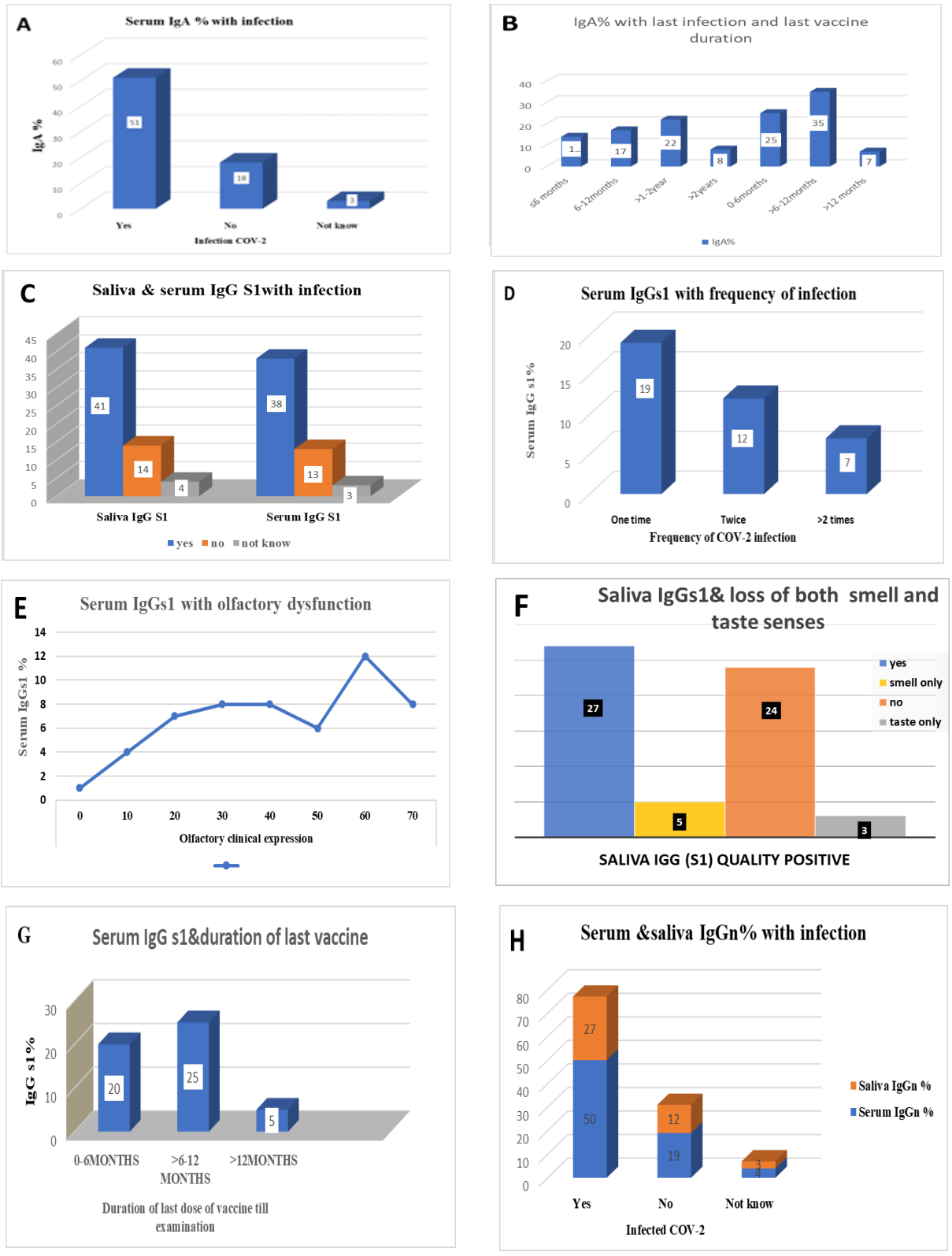

Figure 1: The Antibodies in serum and saliva in relation to other variables. **A**: Serum IgA relation with (+) COVID-19 infection**. B**: Serum IgA relation with last infection and vaccine duration **C**: Serum and saliva IgGs1relation with (+) COVID-19 infction.**D**: Serum IgGs1with frequency of infection.**E**: Serum IgGs1related with olfactory dysfunction scores more lower scores mean ansomia.**F:** Saliva IgGs1 related with loss of both senses smell& taste.**G:** Serum IgGs1 with last vaccine duration.**H:** Serum &saliva IgGn relatd to (+) COVID-19 infection.

The immune cells' relation

The CD4, TH2, and TH1cells percentages had a significant relation to the infected participants (p-value=0.001,0.008,0.008) respectively, as more than half of the tested participants had experienced infection with COVID-19 (21out 36) had 'yes' answers of infected or not? Against 13 had 'No' and 2 "don't know". On the other hand, only CD4 had a significant association with many times of infection (p-value=0.017) as 13 of the 21 infected participants had a one-time infection, 6 had twice infections and only 2 participants had more than twice. The CD4 percentage related to the loss of both senses (p-value=0.009), 14 participants out of 36 had lost both senses,1 lost taste only, and 2 had lost smell only. From the 17 loss senses participants, 11 recovered their sense loss within 2 weeks, 3 recovered within 15 days a month, and 3 recovered within > 1 month the CD4 percentage related to the recovery time (p-value=0.01). The vaccine type related to CD4 (p-value=0.03) 26 participants out of 36 received Pfizer, so the CD4 significantly related to the Pfizer type of vaccine, Figure 3. (B, C, D, E, F).

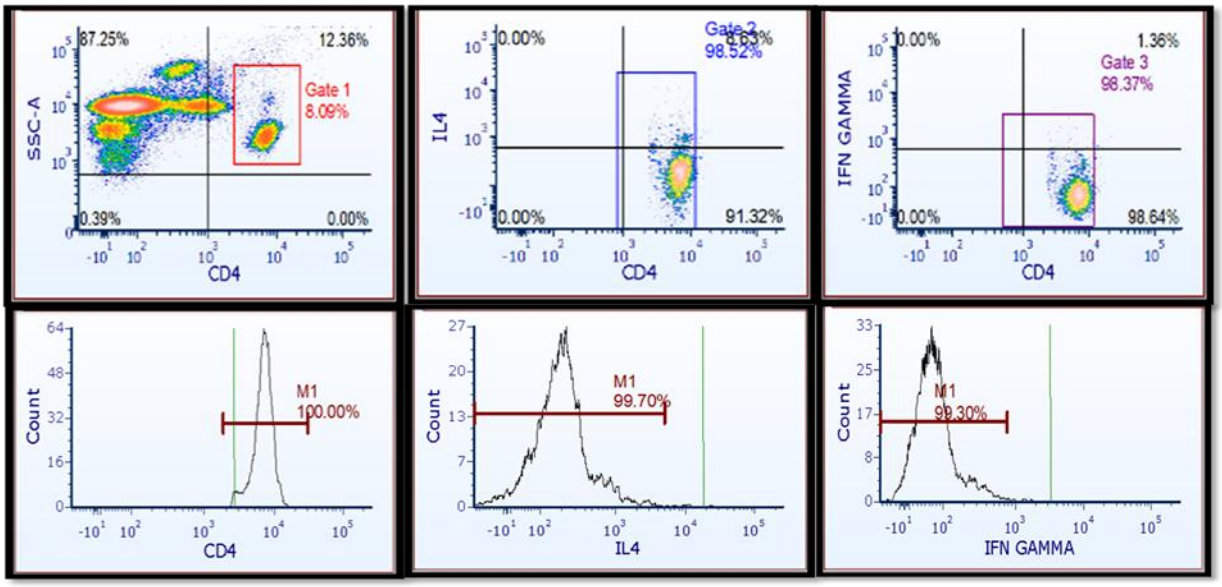

Figure 2: The density plots and histogram of 6 plots for the infected participant

Discussion

The cluster determinant 4 (CD4) molecules express the T cells which contain members of the immunoglobulin and mediates adhesion to the major histocompatibility complex. It proliferates rapidly during acute infection and is exposed to rapid impairment and death. The CD4 count, measured through flow cytometry, is expressed as a percentage. A CD4 percentage between 14% and 28% corresponds to an absolute count of 200 to 500 cells per microliter, while a CD4 percentage below 14% corresponds to an absolute count of fewer than 200 cells per microliter, a classification used by the Centers for Disease Control and Prevention (CDC) in defining AIDS [32].

In this study, the CD4 percentage ranged from 15.76% in infected individuals to 22.24% in unvaccinated individuals. CD4 memory T cells, which may have been induced by prior exposure to common cold human coronaviruses or by the primary COVID-19 vaccination, became specific to SARS-CoV-2. This led to an increase in CD4 T cells during the initial COVID-19 infection and the onset of clinical symptoms [33]. Memory T cells persist in individuals who have recovered from the illness. In severe COVID-19 cases, the number of CD4 T cells specific to COVID-19 decreased, along with reduced production of IL-4 and IFN-ɣ [34]. T-helper lymphocytes start as naive cells (Th0s), which, when activated, differentiate or "polarize" into type 1 (Th1) or type 2 (Th2) lymphocytes [35].

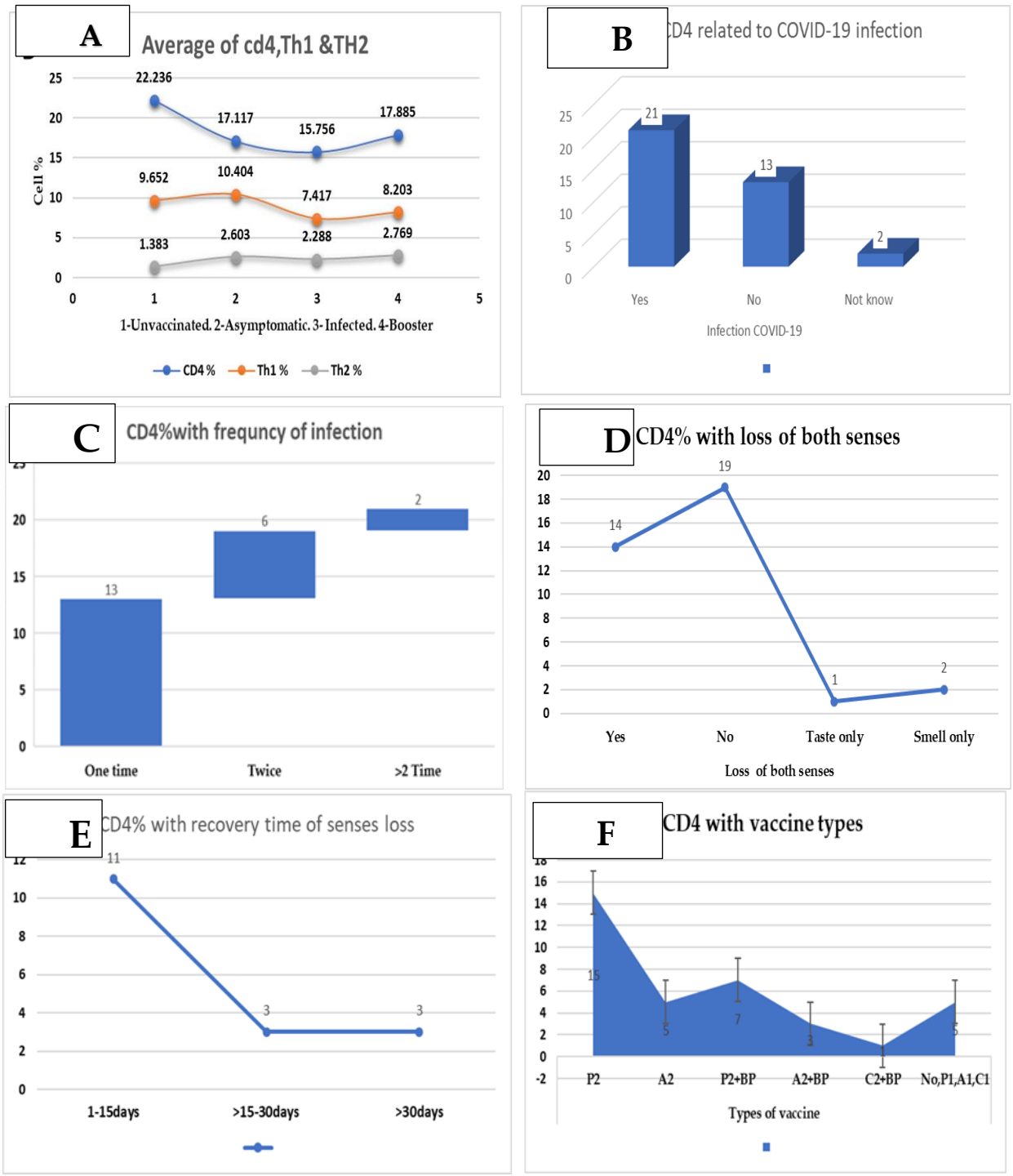

Figure 3: The CD4 relations to many variables, **A-** Average percentage of CD4, Th1, Th2, **B-** CD4 relation positive COVID-19 infection, **C-** The times of infection, **D-** loss of both smell and taste sense, **E-** The relation with recovery time within 15 days, **F-** The relation with Pfizer type of vaccine**.**

The recovered participants in this study had a lower percentage of Th1 than Th2 in all study groups which could be explained by the later stages of recovery, Measuring Th1 cytokines in the early stages of the disease is valuable as an early marker for diagnosing and classifying disease severity [36]. The proportions of Th1 and Th2 cells were higher in patients when the disease was in remission, compared to when they were lower than those in healthy controls [35, 37]. As the disease recovers, the immune system strengthens and better regulates immune responses, leading to a reduction in disease severity [38]. The percentage of all cells was significantly related to infection of COVID-19 since most of the study participants had a history of positive infection of COVID-19. The detection of specific T cells in individuals with a negative

history of COVID-19 infection in the asymptomatic group in this study has been attributed to cross-reaction with peptides from common cold human coronaviruses [39].

The population of CD4 cells within this group of donors had a reduction compared to normal persons in time other than the pandemic this was associated with a reduction in absolute numbers of subpopulations Th1, and Th2 [40]; This could be explained depending on postulated cytopathic effects of the virus that caused direct infection to lymphocyte or suppression of bone marrow by antiviral agents [41]. The CD4 cells positively correlated to Th2 subtypes with a high significant value (person correlation=0.442; p=0.007) at p-value 0.01, on the other hand, a positive non-significant correlation could be found with Th1. The Th1 deficiency may be due to inhibition by elevated levels of Th2 cells and its secreted cytokines like IL-4 [42]. This finding agreed with other studies[40].

CD4 count is significantly associated with a single infection compared to multiple infections, which aligns with the study's suggestion of increased susceptibility to infection. This increased susceptibility may result from an imbalance between Th1 and Th2 cells, as indicated by the findings of this study. loss of both sense of smell and taste as a complication of COVID-19 associated with CD4 levels this could be explained that T-cell mediated inflammation persists in the olfactory epithelium after SARS-CoV-2 has been eliminated from the tissue, suggesting the underlying mechanism of smell loss in post-COVID [43] with Fast recovery within 15 days.

A significant relation between CD4 and Pfizer type of vaccine; this could be explained by that the vaccines can trigger 'antimicrobial resistance' by increasing baseline immunity and can train the immunity to donate protection against the pandemic. Despite all, the booster dose 'which was Pfizer restricted in this study'; can cause reprogramming trained of immune cells at functional levels, epigenetic, and transcription[44]. The study findings agreed with a previous study on recovered individuals following mRNA prime and boost vaccination that induced rapid antigen-specific $CD4^+$ T cell responses and Th1 and Tfh cell responses following the first dose [45]. The study observed that BNT162b2 vaccination had immune responses that exceeded the response of actual COVID-19 infection in specific T cell and antibody levels; the efficacy of >75% as early as 15 to 28 days after primary vaccination [46]. This coincided with this study's findings as a higher percentage of CD4 levels shown in the unvaccinated group as the individual within this group included participants received only a single dose of vaccine whether Pfizer or AstraZeneca This confirmed to get protection from hospitalization (85-94%) for one month period [47].

A positive influence of these cells on the humoral response to COVID-19 especially spike protein serum antibodies was found to correlate with levels of IgG and IgA titers [48]. This could be true in some sort in this study as CD4 levels positively and significantly correlated to serum IgG n but weakly non-significant correlated to IgG s and IgA s& n in both serum and saliva. COVID-19 vaccination promotes long-lasting memory cell production after two doses, independent of previous infection status. The presence of secretory IgA (S IgA) gives the defense mechanisms to mucosal surfaces and can be measured in saliva, Secretory IgA levels showed a highly significant difference in the followed-up group after the first vaccination[49]). IgG and IgA antibodies against the SARS-CoV-2 Spike proteins can be detected in saliva [50] Serum circulation IgA antibodies at 2–3 mg/ml could be detected in a previous study [51]; this study showed higher levels of serum IgA as a general whole participant they showed 3.8 mg/ml regarding the groups the infected group showed a higher level 5.1 mg/ml, this could be due to the inverse correlation between titers of IgA and viral loud after a period of COVID-19 symptoms disappear this could be express in this study as a significant relation found between history of covid infection and IgA titers in serum, as IgA (+) individuals seem to have mild symptoms or they are asymptomatic [52] this was completely true with this study findings as the quantity of IgA S+N in saliva of asymptomatic group higher than that quantity in serum.

Changes in the humoral response following vaccination were noted 15 to 20 days after the first dose and 5 to 7 weeks after the second dose. Specific IgA titers began to decline starting at the third month, with anti-S IgA levels dropping significantly and becoming undetectable by the sixth month following disease

onset in all cases [48]. this finding was unagreed with this study's findings as a significant relation between the last vaccine dose >6-12months and IgA antibody titers and a significant association between these antibody titers and the last infection of covid duration which mostly with a period >1-2 years till the time of examination this could be explained great genetic differences exist in IgA activity between different populations and ethnic variations [53]. Serum IgG is an important antibody in dedicated immunity, acting after innate immunity [51], and persists for more than 12 months after the onset of symptoms, even though their concentrations decline over time. [54].

Some studies suggest that individuals with symptomatic SARS-CoV-2 infections exhibit significantly higher levels of SARS-CoV-2 spike (anti-S) protein antibodies compared to individuals with asymptomatic infections, this could be completely agreed with this study result, a high significant relation was found between the history of COVID-19 infection and the level of IgGs1 in both serum and saliva. The IgG s1 levels were higher in the infected group than asymptomatic group and the level of IgG s1 in both groups was higher in saliva than in serum Besides of level of IgG s in the other two groups booster and unvaccinated groups had a higher level in the the saliva than serum, in all groups of this study in both positive quantity and quality the level of IgGs1 in saliva was higher than serum [55].

These high levels of saliva IgG s1 were associated with loss of both sense smell and taste in samples of this study as it was one of the COVID-19 complications while the serum levels of this IgG were associated with clinical olfactory dysfunction as anosmia complaint individual expressed low level of IgG s1. A significant relation between the level of IgG s1 in serum and times of infection as a high level of this serum antibody is expressed in one-time infection individuals this agreed with study findings showed that younger individuals with asymptomatic COVID-19 had lower levels of IgG antibodies, which could increase their vulnerability to reinfection [54]. On the other hand, a more sustained antibody response was still detectable seven months after acute SARS-CoV-2 infection, indicating that symptomatic cases may lead to higher and longer-lasting antibody concentrations [56]. IgG antibodies targeting the S protein are regarded as protective, making them the main focus in the development of COVID-19 vaccines; this co-incised with a high level of IgG s1 level significantly related to the duration of the last dose of vaccine >6-12months.

A high concentration of IgG against N-protein caused a threefold increase in risk of admission to the ICU. It could be theorized that N protein IgG may favor a higher inflammatory response during infection of COVID-19 [57], this coincided with this study results as the level of IgG n of positive history of COVID-19 infection much higher than the level of IgG s1in serum of the same positive history individuals with high significant relation (p-value=0.000) between this antibodies level and history of COVID-19 infection. The presence of N protein particles on the membranes of infected cells could help explain the process by which natural killer cells, neutrophils, and macrophages interact with IgG antibodies. This interaction contributes to the elimination of infected cells. Furthermore, the formation of immune complexes targeting the N protein may serve as an effective mechanism for clearing the virus [58].

Conclusions

The CD4 cells and all three serum antibodies levels were significantly related to positive COVID-19. Imbalance between Th1 and Th2 increased susceptibility to reinfection with COVID-19. Infection beside the saliva antibodies of both IgG s and n protein were also significantly related to infection. The CD4 cells positively and significantly correlated with IgG n, which was higher related to COVID-19 infection than IgG s. Unlike previous findings the IgA could be detected for a longer time in the serum and saliva of post-COVID-19 individuals regardless of the duration of the last infection or last vaccine.

Limitation of the study

Detection of the amount of INF-ɣ, and IL-4 in the serum of individuals about flow cytometry counting of cell percentage could provide an accurate association and a cut point about the amount of TH1 and TH2 in the blood of post-COVID-19 individuals.

Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

FMA; Conceptualization, Methodology, Investigation, Formal analysis, Software, Writing-original draft and data curation. ARD and MLG; Validation, Supervision, Visualization, Writing-Review & Editing. The results were reviewed by all authors, approving the publication of the final manuscript version.

Acknowledgement and funding

This study didn't receive any financial support from either governmental or private sectors.

Informed consent

All individuals in this study provided an informed consent.

Refferences

1. Centers for Disease Control and Prevention. COVID-19 Testing: What You Need to Know 2022.
2. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med. 2020;26(6):845-8 10.1038/s41591-020-0897-1. https://doi.org/10.1038/s41591-020-0897-1
3. Choe PG, Perera R, Park WB, Song KH, Bang JH, Kim ES, et al. MERS-CoV Antibody Responses 1 Year after Symptom Onset, South Korea, 2015. Emerg Infect Dis. 2017;23(7):1079-84. https://doi.org/10.3201/eid2307.170310
4. Resham AK, Qassem WJ, Mohammad WJ. Evaluation of Nurses' Knowledge and Attitudes about the Prevention of the Coronavirus Disease 2019 at Emergency Units in Government Hospitals in Baghdad City/Iraq. KJNS. 2021;11(2):17-29. https://doi.org/10.36321/kjns.vi20212.2007
5. Zeng W, Liu G, Ma H, Zhao D, Yang Y, Liu M, et al. Biochemical characterization of SARS-CoV-2 nucleocapsid protein. Biochem Biophys Res Commun. 2020;527(3):618-23. https://doi.org/10.1016/j.bbrc.2020.04.136
6. Iyer AS, Jones FK, Nodoushani A, Kelly M, Becker M, Slater D, et al. Dynamics and significance of the antibody response to SARS-CoV-2 infect.. medRxiv. 2020. https://doi.org/10.1101/2020.07.18.20155374
7. Jalkanen P, Pasternack A, Maljanen S, Melén K, Kolehmainen P, Huttunen M, et al. A Combination of N and S Antigens With IgA and IgG Measurement Strengthens the Accuracy of SARS-CoV-2 Serodiagnostics. J Infect Dis. 2021;224(2):218-28. https://doi.org/10.1093/infdis/jiab222
8. Chen Y, Liu Q, Guo D. Emerging coronaviruses: Genome structure, replication, and pathogenesis. J MED VIROL. 2020;92(4):418-23. https://doi.org/10.1002/jmv.25681
9. Dutta NK, Mazumdar K, Gordy JT. The Nucleocapsid Protein of SARS–CoV–2: a Target for Vaccine Development. JVI..2020;94(13). https://doi.org/10.1128/jvi.00647-20
10. McAndrews KM, Dowlatshahi DP, Dai J, Becker LM, Hensel J, Snowden LM, et al. Heterogeneous antibodies against SARS-CoV-2 spike receptor binding domain and nucleocapsid with implications for COVID-19 immunity. JCI Insight. 2020;5(18). https://doi.org/10.1172/jci.insight.142386
11. Muslim Dawood S, Khudhur Al Joofy I. Evaluation of IgM and IgG in COVID-19 Recovered Patients in Iraq. Arch Razi Inst. 2022;77(3):1191-7. https://doi.org/10.22092/ari.2022.357515.2054
12. Ali MA, Hu C, Jahan S, Yuan B, Saleh MS, Ju E, et al. Sensing of COVID-19 Antibodies in Seconds via Aerosol Jet Nanoprinted Reduced-Graphene-Oxide-Coated 3D Electrodes. Adv Mater. 2021;33(7):2006647. https://doi.org/10.1002/adma.202006647
13. Wajnberg A, Amanat F, Firpo A, Altman DR, Bailey MJ, Mansour M, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science..2020;370(6521):1227-30. https://doi.org/10.1126/science.abd7728

14. Interim Guidelines for COVID-19 Antibody Testing in Clinical and Public Health Settings. , CDC, 16 Dec. 2022.

15. Morales-Narváez E, Dincer C. The impact of biosensing in a pandemic outbreak: COVID-19. Biosens. Bioelectron. 2020;163:112274. https://doi.org/10.1016/j.bios.2020.112274

16. Phelan AL. COVID-19 immunity passports and vaccination certificates: scientific, equitable, and legal challenges. Lancet.2020;395(10237):1595-8. https://doi.org/10.1016/s0140-6736(20)31034-5

17. Al Hajji Y, Taylor H, Starkey T, Lee LYW, Tilby M. Antibody response to a third booster dose of SARS-CoV-2 vaccination in adults with hematological and solid cancer: a systematic review. BJC. 2022;127(10):1827-36. https://doi.org/10.1038/s41416-022-01951-y

18. Fendler A, Shepherd STC, Au L, Wilkinson KA, Wu M, Schmitt AM, et al. Immune responses following the third COVID-19 vaccination are reduced in patients with hematological malignancies compared to patients with solid cancer. Cancer Cell. 2022;40(2):114-6. https://doi.org/10.1016/j.ccell.2021.12.013

19. Marlet J, Gatault P, Maakaroun Z, Longuet H, Stefic K, Handala L, et al. Antibody Responses after a Third Dose of COVID-19 Vaccine in Kidney Transplant Recipients and Patients Treated for Chronic Lymphocytic Leukemia. Vaccines. 2021;9(10):1055. https://doi.org/10.3390%2Fvaccines9101055

20. Ali MA, Hu C, Zhang F, Jahan S, Yuan B, Saleh MS, et al. N protein-based ultrasensitive SARS-CoV-2 antibody detection in seconds via 3D nanoprinted, microarchitected array electrodes. J Med Viro. 2022;94(5):2067-78. https://doi.org/10.1002/jmv.27591

21. Shapiro LC, Thakkar A, Campbell ST, Forest SK, Pradhan K, Gonzalez-Lugo JD, et al. Efficacy of booster doses in augmenting waning immune responses to COVID-19 vaccine in patients with cancer. Cancer Cell. 2022;40(1):3-5. https://doi.org/10.1016/j.ccell.2021.11.006

22. Zeng C, Evans JP, Chakravarthy K, Qu P, Reisinger S, Song N-J, et al. COVID-19 mRNA booster vaccines elicit strong protection against the SARS-CoV-2 Omicron variant in patients with cancer. Cancer Cell. 2022;40(2):117-9. (Crossref) https://doi.org/10.1016%2Fj.ccell.2021.12.014

23. Reimann P, Ulmer H, Mutschlechner B, Benda M, Severgnini L, Volgger A, et al. Efficacy and safety of heterologous booster vaccination with Ad26.COV2.S after BNT162b2 mRNA COVID‐19 vaccine in haemato‐oncological patients with no antibody response. Br J Haematol. 2022;196(3):577-84. https://doi.org/10.1111/bjh.17982

24. Fendler A, Shepherd STC, Au L, Wu M, Harvey R, Wilkinson KA, et al. Functional immune responses against SARS-CoV-2 variants of concern after fourth COVID-19 vaccine dose or infection in patients with blood cancer. Cell Rep Med. 2022;3(10):100781. https://doi.org/10.1016%2Fj.xcrm.2022.100781

25. Aleebrahim-Dehkordi E, Molavi B, Mokhtari M, Deravi N, Fathi M, Fazel T, et al. T helper type (Th1/Th2) responses to SARS-CoV-2 and influenza A (H1N1) virus: From cytokines produced to immune responses. Transpl Immunol. 2022;70:101495. https://doi.org/10.1016/j.trim.2021.101495

26. Raphael I, Joern RR, Forsthuber TG. Memory CD4(+) T Cells in Immunity and Autoimmune Diseases. Cells. 2020;9(3). https://doi.org/10.1016/j.trim.2021.101495

27. Le Bert N, Tan AT, Kunasegaran K, Tham CYL, Hafezi M, Chia A, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. Nature. 2020;584(7821):457-62. https://doi.org/10.1038/s41586-020-2550-z

28. Adamo S, Chevrier S, Cervia C, Zurbuchen Y, Raeber ME, Yang L, et al. Lymphopenia-induced T-cell proliferation is a hallmark of severe COVID-19. 2020. https://doi.org/10.1101/2020.08.04.236521

29. Kuri-Cervantes L, Pampena M, Meng W, Rosenfeld A, Ittner C, Weisman A, et al. Comprehensive mapping of immune perturbations associated with severe COVID-19. Sci. Immunol. 2020;5:eabd7114. https://doi.org/10.1126/sciimmunol.abd7114

30. Koch T, Mellinghoff SC, Shamsrizi P, Addo MM, Dahlke C. Correlates of Vaccine-Induced Protection against SARS-CoV-2. Vaccines. 2021;9(3):238. https://doi.org/10.3390/vaccines9030238

31. Neidleman J, Luo X, Frouard J, Xie G, Gill G, Stein ES, et al. SARS-CoV-2-Specific T Cells Exhibit Phenotypic Features of Helper Function, Lack of Terminal Differentiation, and High Proliferation Potential. Cell Rep Med. 2020;1(6):100081. https://doi.org/10.1016/j.xcrm.2020.100081

32. Gil-Etayo FJ, Suàrez-Fernández P, Cabrera-Marante O, Arroyo D, Garcinuño S, Naranjo L, et al. T-Helper Cell Subset Response Is a Determining Factor in COVID-19 Progression. Front Cell Infect Microbiol. 2021;11:624483. https://doi.org/10.3389/fcimb.2021.624483

33. Rahimzadeh M, Naderi N. Toward an understanding of regulatory T cells in COVID-19: A systematic review. J Med Virol. 2021;93(7):4167-81. https://doi.org/10.1002/jmv.26891

34. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. JCE. 2008;61(4):344-9. https://doi.org/10.1016/j.jclinepi.2007.11.008

35. Li; R, Duffee; D, Gbadamosi-Akindele. MF. CD4 Count. National library of health: StatPearls Publishing; 2023 Last Update: May 1, 2023.

36. Salomé B, Horowitz A. Impaired CD4 T-cell response to SARS-CoV-2: rationale for PD-1 blockade in patients with cancer and COVID-19? Cancer Discov.2021;11(8):1877-8. https://doi.org/10.1158/2159-8290.CD-21-0613

37. Oja AE, Saris A, Ghandour CA, Kragten NA, Hogema BM, Nossent EJ, et al. Divergent SARS-CoV-2-specific T-and B-cell responses in severe but not mild COVID-19 patients. Eur. J. Immunol. 2020;50(12):1998-2012. https://doi.org/10.1002/eji.202048908

38. Supriya R, Gao Y, Gu Y, Baker JS. Role of Exercise Intensity on Th1/Th2 Immune Modulations During the COVID-19 Pandemic. Frontiers in Immunology. 2021;12. https://doi.org/10.3389/fimmu.2021.761382

39. Adnan Mezher M, Bahjat Alrifai S, Mahmood Raoof W. Analysis of Proinflammatory Cytokines in COVID-19 Patients in Baghdad, Iraq. Archives of Razi Institute. 2023;78(1):305-13. https://doi.org/10.22092%2FARI.2022.359356.2411

40. McGeachy MJ, Cua DJ. T cells doing it for themselves: TGF-β regulation of Th1 and Th17 cells. Immunity. 2007;26(5):547-9. https://doi.org/10.1016/j.immuni.2007.05.003

41. Fathi F, Sami R, Mozafarpoor S, Hafezi H, Motedayyen H, Arefnezhad R, et al. Immune system changes during COVID-19 recovery play a key role in determining disease severity. Int J Immunopathol Pharmacol. 2020;34:2058738420966497.

42. Cassaniti I, Percivalle E, Bergami F, Piralla A, Comolli G, Bruno R, et al. SARS-CoV-2 specific T-cell immunity in COVID-19 convalescent patients and unexposed controls measured by ex vivo ELISpot assay. Avicenna J Clin Microbiol Infect. 2021;27(7):1029-34. https://doi.org/10.1016/j.cmi.2021.03.010

43. Juan Francisco Gutiérrez-Bautista, Antonio Rodriguez-Nicolas, Antonio Rosales-Castillo, Pilar Jiménez, Federico Garrido, Per Anderson, et al. Negative Clinical Evolution in COVID-19 Patients Is Frequently Accompanied With an Increased Proportion of Undifferentiated Th Cells and a Strong Underrepresentation of the Th1 Subset 2020;11. https://doi.org/10.3389/fimmu.2020.596553

44. Li H, Liu L, Zhang D, Xu J, Dai H, Tang N, et al. SARS-CoV-2 and viral sepsis: observations and hypotheses. The Lancet. 2020;395(10235):1517-20. https://doi.org/10.1016/s0140-6736(20)30920-x

45. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the Cytokine Storm in COVID-19. J Infect.. 2020;80(6):607-13. https://doi.org/10.1016/j.jinf.2020.03.037

46. Finlay JB, Brann DH, Abi Hachem R, Jang DW, Oliva AD, Ko T, et al. Persistent post-COVID-19 smell loss is associated with immune cell infiltration and altered gene expression in olfactory epithelium. Sci Transl Med. 2022;14(676):eadd0484. https://doi.org/10.1126/scitranslmed.add0484

47. Mantovani A, Netea MG. Trained innate immunity, epigenetics, and Covid-19. N Engl J Med Overseas Ed. 2020;383(11):1078-80.

48. Painter MM, Mathew D, Goel RR, Apostolidis SA, Pattekar A, Kuthuru O, et al. Rapid induction of antigen-specific CD4(+) T cells is associated with coordinated humoral and cellular immunity to SARS-CoV-2 mRNA vaccination. Immunity. 2021;54(9):2133-42.e3. https://doi.org/10.1016/j.immuni.2021.08.001

49. Amit S, Regev-Yochay G, Afek A, Kreiss Y, Leshem E. Early rate reductions of SARS-CoV-2 infection and COVID-19 in BNT162b2 vaccine recipients. The Lancet. 2021;397(10277):875-7. https://doi.org/10.1016/s0140-6736(21)00448-7

50. Supriya R, Gao Y, Gu Y, Baker JS. Role of exercise intensity on Th1/Th2 immune modulations during the COVID-19 pandemic. Front Immunol. 2021;12:761382. https://doi.org/10.3389/fimmu.2021.761382

51. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell. 2020;181(7):1489-501. e15. https://doi.org/10.1016/j.cell.2020.05.015

52. Ali D, Taha G. Effect of Covid-19 vaccine on some immunological salivary biomarkers (sIgA and Interleukine-17). J Fac Med Baghdad. 2023;65(2). https://doi.org/10.32007/jfacmedbagdad.2039

53. Sheikh-Mohamed S, Isho B, Chao GYC, Zuo M, Cohen C, Lustig Y, et al. Systemic and mucosal IgA responses are variably induced in response to SARS-CoV-2 mRNA vaccination and are associated with protection against subsequent infection. Mucosal Immunol. 2022;15(5):799-808. https://doi.org/10.1038/s41385-022-00511-0

54. Barzegar-Amini M, Mahmoudi M, Dadgarmoghaddam M, Farzad F, Najafabadi AQ, Jabbari-Azad F. Comparison of Serum Total IgA Levels in Severe and Mild COVID-19 Patients and Control Group. J Clin Immunol. 2022;42(1):10-8. https://doi.org/10.1007%2Fs10875-021-01149-6

55. Rangel-Ramírez VV, Macías-Piña KA, Servin-Garrido RR, de Alba-Aguayo DR, Moreno-Fierros L, Rubio-Infante N. A systematic review and meta-analysis of the IgA seroprevalence in COVID-19 patients: Is there a role for IgA in COVID-19 diagnosis or severity? Microbiol Res. 2022;263:127105. https://doi.org/10.1016/j.micres.2022.127105

56. Watanabe S, Naito Y, Yamamoto T. Host factors that aggravate COVID-19 pneumonia. Int J Fam Med Prim Care. 2020; 1 (3). 2020;1011.

57. Carvalho Á, Henriques AR, Queirós P, Rodrigues J, Mendonça N, Rodrigues AM, et al. Persistence of IgG COVID-19 antibodies: A longitudinal analysis. Front Public Health. 2022;10:1069898. https://doi.org/10.3389/fpubh.2022.1069898

58. Boyton RJ, Altmann DM. The immunology of asymptomatic SARS-CoV-2 infection: what are the key questions? Nat Rev Immunol. 2021;21(12):762-8. https://doi.org/10.1038/s41577-021-00631-x

تقييم المناعة الخلوية والخلطية لدى اطباء الاسنان العراقيين المتعافين من كوفيد

فريال محمود عبد الرضا, د.امينة ريحان دعيجل Michaela Louise Goodson

المستخلص:

الخلفية: اطباء الاسنان، اثناء مواجهتهم للامراض المعدية، يقعون في خطر العدوى هذه الدراسة تهدف لتقييم المناعى بعد الاصابة بكوفيد 19، من خلال العوامل الحيوية في اللعابو الدم التي تساعد على التنبئ بالحالة الصحية ومتابعة الحالة المناعية اختيار العينة ونوع للدراسة دراسة مستعرضة تمت على 91 طبيب اسنان عراقي عاملين بين حزيران وآب في عام 2022 في اللعاب ومصل الدم لكل المشاركين واختيار 36مشارك من ال91 عشوابيا لغرض قياس نسبة IgG, IgA لكلا s1+nالطريقة باستخدام عدة الايلايزا الخاصة بالكوفيد 19 2TH 2 كانت منخفضة، حيث ان مستوى 1Th و 4Th و 2CD في كامل الدم يوميا النتائج كل مستويات نسب CD4,Th1,Thالخلايا المناعية من خلال جهاز الفلوسيتومیتر لقياس 4 كانت متعلقة بلقاح نوع فايزر، خسارة كلتا الحاستين 1CD في كل المشاركين كل الخلايا كانت مصاحبة بشكل كبير بماض من العدوى بكوفيد 19 بينما THكان عاليا وثبط مستويات داخل الدم؛ هذا الجسم المضاد ذو اهمية عالية في حالات العدوى بكوفيد n نوع 4IgG مع 2CD و 4Th مع CDالشم والذوق والتعافي خلال فترة 15 يوم هنالك علاقة ايجابية وجدت بين داخل الدم كانت، بشكل ملحوظ، متعلقة بتاريخ من العدوى وقد تكون قابلة للملاحظة في الاشخاص الذين عانوا من (s+n 1IgA داخل الدم مستويات s نوع 19IgG، اعلى من مستويات 2 جعلا الاشخاص المتعافين اكثر عرضة لعودة الاصابة 4Th1/Th وعدم توازن في نسبة CDالعدوى منذ 1 2 سنة واخذ لقاح منذ 6 12 شهر الاستنتاج مستوى نسبة منخفض من بمتحورات جديدة لكن النسبة العالية للاجسام المضادة الخاصة بكوفيد التي تلت الاصابة الوحيدة بالعدوى او اخذ جرع تذكيرية من اللقاح منحتهم حماية.

.