doi.org/10.36721/PJPS.2023.36.4.REG.1031-1043.1

Clinical importance of zinc as monotherapy in modulating RT-PCR cycle threshold values and antibody levels in cases of COVID 19 patients

Maha Ibrahim[1], Widad M Al-Bishri[2], Hanan S Alnahdi[2], Samia M. Ahmed[3] and Ammar Bayoumi[4]

[1]Department of Hematology, MSF for Medical Research and Development, Jeddah, Saudi Arabia
[2]Department of Biochemistry, College of Science, University of Jeddah, Jeddah, Saudi Arabia
[3]Department of Medical Laboratory Technology, Faculty of Applied Medical Science, Taibah University, Saudi Arabia
[4]Department of Microbiology, MSF for Medical Research and Development, Jeddah, Saudi Arabia

Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is globally concerning for overall health. The viral burden is diagnosed by the positive cycle threshold value (Ct-value) of the real-time reverse transcription polymerase chain reaction (RT-PCR) assay. So far, no effective therapy has been established for this viral infection. This research aims to investigate the impact of zinc therapy on viral burden, salivary zinc levels and serum specific antibody levels versus SARS-CoV-2 spike antigen in subjects with infection. The correlation between viral burden and salivary zinc levels was also studied. 75 participants were included, classified as 25 non SARS-CoV-2 healthy individuals, 25 SARS-CoV-2 patients and 25 SARS-CoV-2 patients receiving zinc sulphate daily for 30 days. Results revealed markedly low salivary zinc levels in SARS-CoV-2 cases, which were closely linked with a high viral burden versus healthy participants. Marked elevations in serum IgM, IgG, and IgG1 antibody levels in infected patients versus healthy participants were also noticed. Treatment with zinc markedly boosted the salivary zinc levels and lowered the viral burden in SARS-CoV-2 cases. Serum IgM, IgG and IgG1 antibody levels were downregulated in SARS-CoV-2 treated with zinc. Conclusion: Zinc therapy may be an efficient therapeutic approach for SARS-CoV-2 viral eradication.

Keywords: SARS-CoV-2, viral burden, zinc sulphate, antibody.

INTRODUCTION

A new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for coronavirus illness 2019 (COVID-19), has developed as a deadly virus and triggered a global pandemic. The infection initially originated in China in December of 2019 and has since spread globally with millions of confirmed cases (Guan *et al.*, 2020). The viral infection can fluctuate in severity from mild cases, which account for more than eighty percent of proven cases, to serious cases with respiratory problems that require the support of a critical care unit (Guan *et al.*, 2020).

Viral burden can be estimated by nucleic acid amplification of SARS-CoV-2 genome based on the cycle threshold value (Ct-value) of real-time reverse transcription polymerase chain reaction (rRT-PCR) that is measured in oropharyngeal or nasopharyngeal specimens. A high viral burden is indicated by a low Ct value (<30). (Zou *et al.*, 2020). Ingberg *et al*. (2022) have illustrated that a low Ct value is correlated with infection severity and is helpful in identifying patients at risk for developing a serious illness. Although low Ct values may be predictive of worse consequences for symptomatic individuals, low Ct are also seen in asymptomatic ones, who represent about 30% of SARS-CoV-2 infected cases (Walsh *et al.*, 220).

A serological or antibody test is another method widely utilized for the detection of specific antibodies versus viral infection instead of the virus itself (Theel *et al.*, 2020, Bastos *et al.*, 2020). SARS-CoV-2 contains four distinct structural proteins that the immune system can recognize as antigens, namely the nucleocapsid (N), spike (S), membrane (M) and envelope (E) (Kontou *et al.*, 2020). N and S proteins are the main proteins accountable for triggering the antibody production (To *et al.*, 2020). The S molecule is crucial for viral fusion and intracellular invasion, while the N molecule aids the viral RNA to bind and pack into a helical nucleocapsid structure during viral replication. Therefore, different investigations have chosen specific antibodies (IgM, IgG and IgA) for S or N proteins to diagnose SARS-CoV-2 (Li and Li, 2020). IgM and IgA are generated at the beginning of viral invasion by host immune cells, while IgG is produced at a later stage (Ishay *et al.*, 2020).

There is insufficient evidence concerning the early treatment of SARS-CoV-2 invasion to mitigate symptom progression. Several research groups have made many substantial attempts to establish SARS-CoV-2 vaccines to immunize people in various countries via national immunization plans (Dhama *et al.*, 2022). However,

*Corresponding author: e-mail: walbeshri@uj.edu.sa

various mutations have been recognized in SARS-CoV-2 versions that may interfere with vaccination-induced antibody responses, resulting in a loss of efficiency (Dhawan *et al.*, 2022). As a result, finding a different treatment plan is therefore essential to lessen any additional SARS-CoV-2 cases.

Earlier studies demonstrated that treatment with particular micronutrients, such as vitamins and zinc, might be useful for reducing the SARS-CoV-2 viral burden and hospitalization time. This may improve treatment approaches to manage the prevalence of viral invasion (Gombart *et al.*, 2020; Balboni *et al.*, 2022).

Zinc has recently been acknowledged as one of the essential minerals for providing supportive treatment for SARS-CoV-2 invasion due to its antiviral and immunomodulatory capabilities (Rahman and Idid, 2021). Previous publication reported that high-dose zinc therapy significantly reduced SARS-CoV-2 patients' symptoms (Finzi, 2020). According to Shakoor *et al*. (2021), zinc can inhibit viral binding and replicating abilities. This can aid in the reduction of the viral burden in severe viral infection cases. Zinc has essential functions in immune cell development and functionality and its deficiency could drastically impair both adaptive and innate immune reactions and increase the vulnerability to viral invasion (Helge and Lothar, 2003; Prasad, 2008). Zinc deficiency can affect antibody production via suppressing B lymphocytes' growth inside the bone marrow, leading to a poor prognosis during the viral invasion (Bonaventura *et al.*, 2015; Pal *et al.*, 2021).

The previous information has demonstrated the critical role of zinc versus SARS-CoV-2 invasion, but the correlation of viral burden with zinc deficiency as well as the immunological response in subjects with SARS-CoV-2 is questionable. The goal of this research is to evaluate the correlation of viral burden (indicated by RT-PCR-Ct values) with salivary zinc levels in SARS-CoV-2 subjects to explore whether zinc insufficiency could be a prognostic marker of infection seriousness. In addition, the correlation of viral burden with viral spike protein specific antibodies (IgM, IgG and IgG1) in infected patients was investigated. This research also investigated the influence of zinc therapy on modulating SARS-CoV-2 viral burden, salivary zinc levels and serum levels of viral spike protein specific antibodies in SARS-CoV-2 infected individuals.

MATERIALS AND METHODS

Participants

This investigation was accepted by the Saudi Ministry of Health (MOH); approval number is A00981. 75 participants in the study were classified into three groups, each of 25 participants, as follows: group 1: healthy non-infected individuals (non-SARS-CoV-2); group 2: SARS-CoV-2 infected patients; and group 3: SARS-CoV-2 +zinc, treated with zinc sulphate capsules (220 mg each containing 50mg zinc, PlusPharma, California, United States) two times daily for one month after a positive diagnosis. According to MOH guidelines, SARS-CoV-2 subjects were classified as severe cases because they required the intervention of a critical care unit. The infected patients were chosen from regional government hospitals in Jeddah, Saudi Arabia, including East Jeddah General Hospital and King Fahad General Hospital. This investigation was performed between September 1, 2020 and September 1, 2021. Before starting the study, each contestant signed an informed consent form.

Inclusion criteria

All involved individuals were unimmunized and had no chronic diseases. Healthy individuals were not formerly infected with the coronavirus. Non-infected individuals were confirmed by high RT-PCR Ct values (>30). Patients with viral infection were ensured by low RT-PCR Ct values (<30). The RT-PCR was carried out utilizing nasopharyngeal swabs.

Exclusion criteria

Subjects immunized against COVID-19 or below 18 years old were excluded. Individuals with chronic diseases, autoimmune illnesses, periodontal inflammation, oral mucosal disorders, or oral cancer were also ruled out.

Collection of demographic data

Demographic information for each contestant (age, country, gender and history of any chronic disease) was recorded via a created form utilizing Survey Monkey website and sent to each patient's WhatsApp number.

Real-time polymerase chain reaction (RT-PCR) and Ct values

SARS-CoV-2 RNA was detected in nasopharyngeal specimens of viral infected patients by RT-PCR. Flocked swabs were utilized to obtain nasopharyngeal biopsies from all individuals, which were then mixed with 0.9% NaCl and inactivated by heating. Viral RNA was extracted utilizing an extraction kit for nucleic acid (Taiwan Advanced Nanotech, Taiwan) in accordance with the instructions of the manufacturer. Specimens were then analyzed utilizing RT-PCR for viral RNA-dependent RNA polymerase (RdRp) gene detection. The reaction was carried out for 30 cycles and Ct values below 30 were regarded as positive.

Salivary zinc determination

All individuals were requested to refrain from eating, smoking, drinking and maintaining dental hygiene for at least one hour prior to the collection of saliva. Utilizing the passive drooling technique, saliva specimens were obtained in the morning between 7 and 9 a.m. About 2ml of saliva specimens were taken and centrifuged at 4°C for

10 min at 10,000 g to get rid of cellular debris. The supernatants were diluted (1: 7) with deionized water and then used to measure zinc concentrations utilizing an atomic absorption spectrophotometer (Varian Techtron Pty. Ltd., Melbourne, Australia) (Hallmans, 1978). The standard solutions (0.1-2µg/ml of zinc) and the zinc samples were aspirated and the optical density was determined at 213.9 nm. Zinc concentrations (µg/ml) were calculated from the standard curve (Chernecky and Berger, 2008).

Collection of blood and serum separation

Blood specimens (5ml) were collected from all participants in different groups and centrifuged for 10 minutes after blood coagulation at 3000rpm for serum separation. The serum samples were then used to estimate specific SARS-CoV-2 anti-spike antibodies.

Determination of SARS-CoV-2 anti-spike antibodies

Antibody estimation was carried out for IgM, IgG and IgG1 versus SARS-CoV-2 spike antigen utilising an indirect SARS-CoV-2 ELISA Kit (Elabscience, United States) according to the procedures of the manufacturer. Concisely, 10 µL of diluted serum specimens beginning at 1:20 for IgM and 1:50 for IgG and IgG1 were placed in 96-well ELISA plates previously coated with purified antigen of SARS-CoV-2 Spike protein, then placed in a shaking incubator at 37°C (Gallenkamp, Germany) for 30 min. After washing, 100 µL of horseradish peroxidase (HRP)-labeled anti-human antibodies, comprising anti-IgM, anti-IgG and anti-IgG1 (1:10,000 each), were applied and incubated for twenty minutes at 37°C. After elimination of free components by washing, 50 µL of substrate (O-phenylenediamine, Sigma-Aldrich, USA) was applied and then left to incubate for ten minutes in the dark at 37°C for colour development. Sulfuric acid (50µL, 1M) was utilized to terminate the enzyme-substrate interaction and the absorbency was recorded at 450nm.

STATISTICAL ANALYSIS

The results were analyzed by utilizing SPSS version 22. Results were calculated as mean ± standard deviation (SD) and the group variables were given as numbers and percentages (%). An unpaired t test was utilized for comparing two means, while chi-square was achieved for comparing the frequency of two groups or more. The strength of a relationship between two variables was evaluated utilizing the Pearson's correlation test. $P<0.05$ is the measure of significance.

RESULTS

Demographic characteristics of different groups

Table 1 shows baseline characterization of study participants. A total of 75 participants with nationality of Saudi Arabian were involved in this investigation (43 males and 32 females). The participants were classified into three groups, each of 25 participants, healthy non-SARS-CoV-2 subjects with no infection, SARS-CoV-2 subjects with severe illness and zinc-SARS-CoV-2 treated subjects. Most subjects in non- SARS-CoV-2 group were females (56.0%), however males were predominated in the SARS- CoV-2 group (64.0%) and zinc–SARS-CoV-2 treated one (64.0%). There were significant difference in age between different studied groups (P=0.018), most healthy individuals were between the ages of 41 and 60 (56%) , while most subjects in SARS-CoV-2 infected group and zinc- SARS-CoV-2 treated one were between the ages of 20 and 40 (64%).

Fig. 1 shows a considerable depletion in Ct values in SARS-CoV-2 subjects versus healthy non- SARS-CoV-2 ones (P≤ 0.001). SARS-CoV-2 subjects who received zinc twice daily for 30 days had significantly higher RT-PCR-Ct values than SARS-CoV-2 untreated ones (P≤ 0.001).

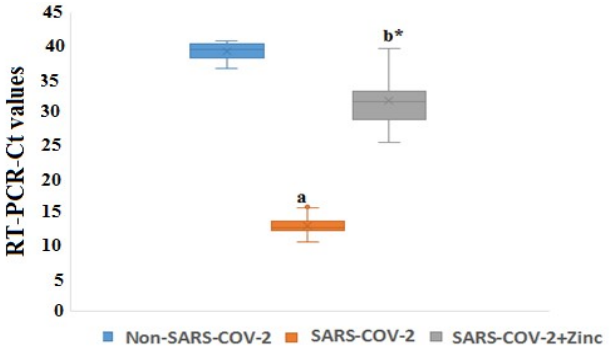

Fig. 1: RT-PCR –Ct values in SARS-CoV-2 subjects (n= 25) and SARS-CoV-2 subjects treated with zinc (n= 25) with relation to non-SARS-COV-2 ones (n= 25). Boxes reveal the range of median and interquartile. [a]P≤ 0.001, [b]P≤ 0.01 versus non- SARS-CoV-2 subjects, *P ≤0.001 versus SARS-CoV-2 subjects.

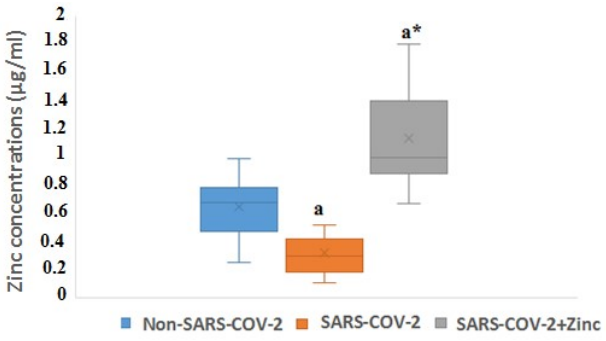

Fig. 2: Salivary zinc levels in SARS-CoV-2 subjects (n= 25) and SARS-CoV-2 subjects treated with zinc (n= 25) with relation to non- SARS-CoV-2 ones (n= 25). Boxes reveal the range of median and interquartile. [a]P≤0.001 versus non- SARS-CoV-2 subjects, *P≤0.001 versus SARS-CoV-2 infected subjects.

Table 1: Demographic characteristics of different groups

Characteristic	Frequency (*n* and %)			*p* value
	Non- SARS-CoV-2 (*n*= 25)	SARS-CoV-2 infected	Zinc - SARS-CoV-2 treated	
Nationality Saudi Arabian Non-Saudi Arabian	25 (100%) 0 (0%)	25 (100%) 0 (0%)	25 (100%) 0 (0%)	
Gender Male Female	11 (44%) 14 (56%)	16 (64.0%) 9 (36.0%)	16 (64.0%) 9 (36.0%)	P=0.256
Age (years) <20 20-40 41-60 61-80	0 (0%) 10 (40%) 14 (56%) 1 (4%)	3 (12%) 16 (64%)* 5 (20) ** 1 (4)	0 (0%) 16 (64%)* 8(32%)** 1(4%)	P= 0.01 *P ≤ 0.01 **P ≤ 01

Data are given as frequency (*n*) and percentage (%). P: significance between groups, *P, **P versus Non- SARS-CoV-2 RT-PCR-Ct values in different participant groups

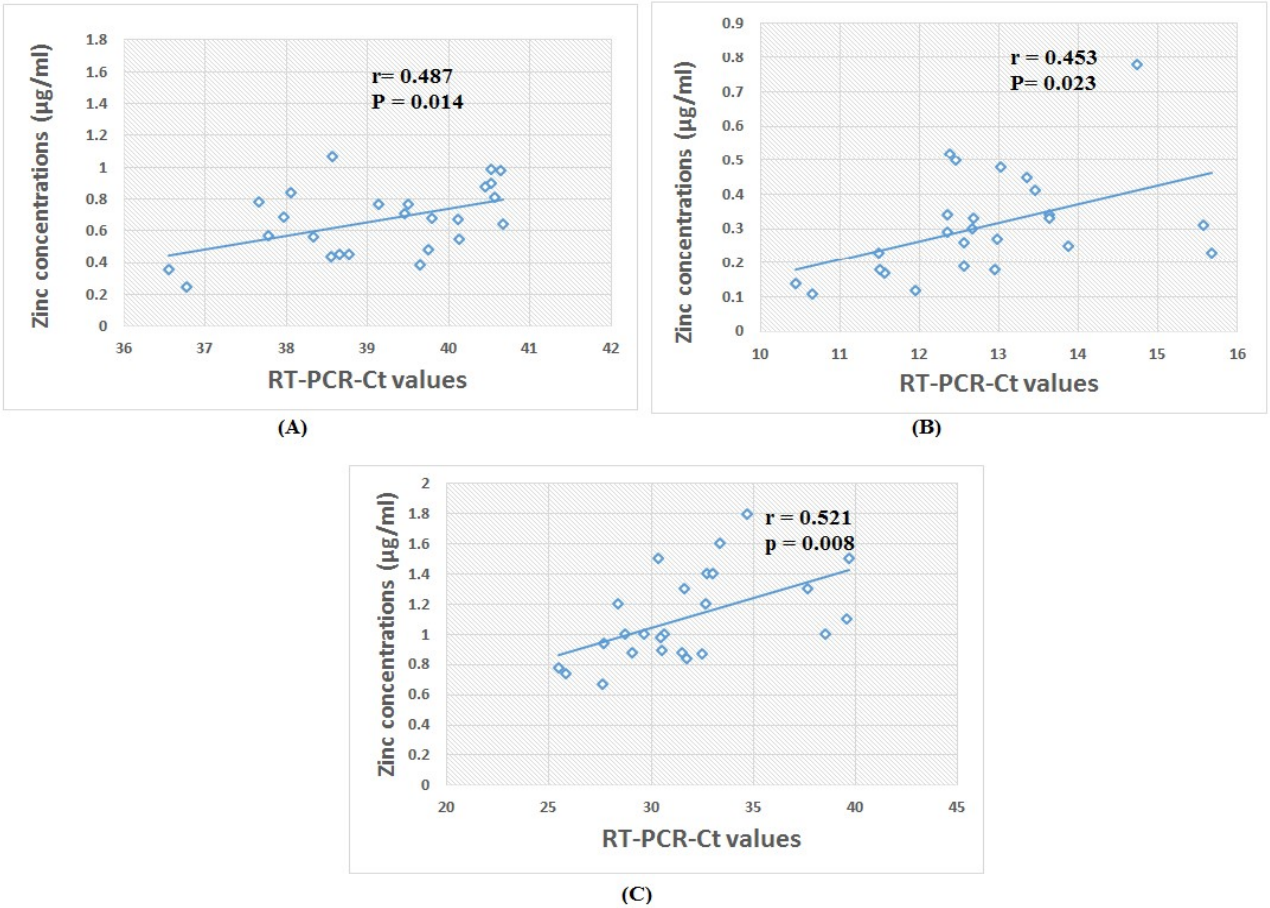

Fig. 3: Correlation between salivary zinc concentrations and RT-PCR-Ct values in various groups. (A) non- SARS-CoV-2 subjects (n=25), (B) SARS-CoV-2 infected subjects (n=25) and (C) SARS-CoV-2 infected subjects treated with zinc (n=25).

Levels of salivary zinc in different groups at various gender and age range.

Fig. 2 demonstrates markedly lower salivary zinc levels in SARS-COV2 infected patients after the onset of symptoms compared with healthy non-SARS-CoV-2 participants (P≤ 0.001). SARS-CoV-2 subjects who received zinc therapy had markedly higher salivary zinc levels than SARS-CoV-2 subjects who did not get zinc therapy (P≤ 0.001).

Correlation between salivary zinc levels and RT-PCR-Ct values in different participant groups.

Pearson's correlation (fig. 3, A-C) revealed a remarkable positive association between salivary zinc concentrations and RT-PCR-Ct values in non- SARS-CoV-2 healthy subjects (r = 0.487, P = 0.014, fig. 3A), SARS-CoV-2 subjects (r = 0.453, P = 0.023, fig. 3B) and SARS-CoV-2 subjects treated with zinc (r = 0.521, P = 0.008, fig. 3C).

Factors affecting salivary zinc concentrations

A-Gender

Statistical analysis of the data (fig. 4).revealed significantly lower salivary zinc levels in females than in males within each studied group, including healthy non-SARS-CoV-2 subjects (P≤0.001), SARS-CoV-2 infected subjects (P≤0.01) and SARS-CoV-2 infected subjects treated with zinc (P≤0.0001).

B-Age

The majority of healthy subjects have age ranges of 40 and 60 (56%), while most of the subjects in the SARS-CoV-2 group and zinc-SARS-CoV-2 group have age ranges of 20 and 40 (64%) (table 1). There were no appreciable variations in the salivary zinc contents between the age classes (20–40 and 40–60 years) within each studied group (fig. 5).

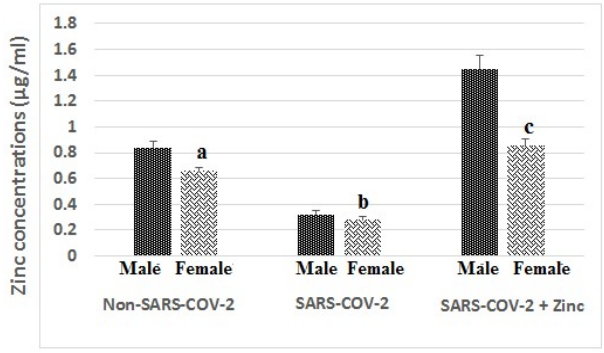

Fig. 4: The impact of gender on salivary zinc concentrations measured in different groups, [a]P≤0.001 versus males in non- SARS-CoV-2 subjects, [b]P≤0.01 versus males in SARS-CoV-2 subjects, [c]P≤0.0001 versus males in SARS-CoV-2 subjects after treatment with zinc. Values were graphed as histograms with mean ± SD.

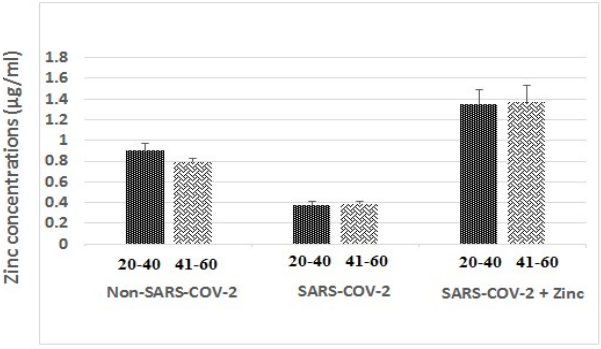

Fig. 5: The impact of age on salivary zinc concentrations measured in different groups. Values were graphed as histograms with mean ± SD. Non-significant variations were noticed in zinc contents between ages 20-40 and 41-60 within each group.

Correlation between salivary zinc concentrations and age

Pearson's correlation showed non-significant negative association between age and salivary zinc concentrations in non- SARS-CoV-2 subjects (r= -0.02, P= 0.92) (fig. 6A) and SARS-CoV-2 infected ones (r= -0.002, P= 0.99) (fig. 6B). A moderate non-significant positive association between age and salivary zinc concentrations was recorded in zinc-SARS-CoV-2 treated subjects (r= 0.32, P= 0.117) (fig. 6C).

SARS-CoV-2 spike -specific antibody responses

The serum concentrations of specific antibodies against SARS-CoV-2 spike antigen are illustrated in fig. 7. The data revealed a significant elevation in the concentrations of IgM, IgG and IgG1 antibodies in SARS-CoV-2 subjects versus healthy individuals (P≤0.001). There were significant decreases in these antibodies in SARS-CoV-2 subjects after treatment with zinc for 30 successive days in comparison to infected untreated ones (P≤0.001).

Pearson's correlation of viral spike specific antibody levels with viral burden

A significant strong positive relationship was identified between IgM concentrations and the clinical RT-PCR-Ct values in non- SARS-CoV-2 healthy individuals (r=0.586, P=0.002, fig. 8A), whereas this relation was non-significant in SARS-CoV-2 subjects (r=0.193, P = 0.356, fig. 8B) and SARS-CoV-2 subjects treated with zin (r= -0.0106, P=0.613, fig. 8C). IgG exhibited weak inverse relation with RT-PCR-Ct values in non SARS-CoV-2 healthy individuals (r= -0.13, P=0.54, fig. 9A), while IgG1 revealed a significant strong negative connection with RT-PCR-Ct values in the same healthy individuals (r= -0.886, P≤0.001, fig. 10A). A non-significant inverse relationship was identified between IgG levels and RT-PCR-Ct values in SARS-CoV-2 subjects (r= -0.33, P= 0.140, fig. 9B), while IgG1 showed a non-significant positive correlation with these values in the same SARS-CoV-2 subjects (r= 0.151, P= 0.471, fig. 10B). In zinc - SARS-CoV-2 treated individuals, there was a significant moderate inverse relation between IgG concentrations and RT-PCR-Ct values (r= -0.50, P= 0.013, fig. 9C), but IgG1 showed a non-significant positive correlation with RT-PCR-Ct values (r= 0.047, P= 0.82, fig. 10C).

Pearson's correlation of viral spike specific antibody levels with salivary zinc concentrations

Serum IgM revealed non-significant weak negative correlation with salivary zinc concentrations in non-SARS-CoV-2 healthy individuals (r= - 0.201, P= 0.347, fig. 11A), while non-significant weak positive correlation was observed between these two parameters in SARS-CoV-2 subjects (r= 0.182, P= 0.384, fig. 11B) and SARS-CoV-2 subjects treated with zinc (r= 0.211, P= 0.322, fig. 11C).

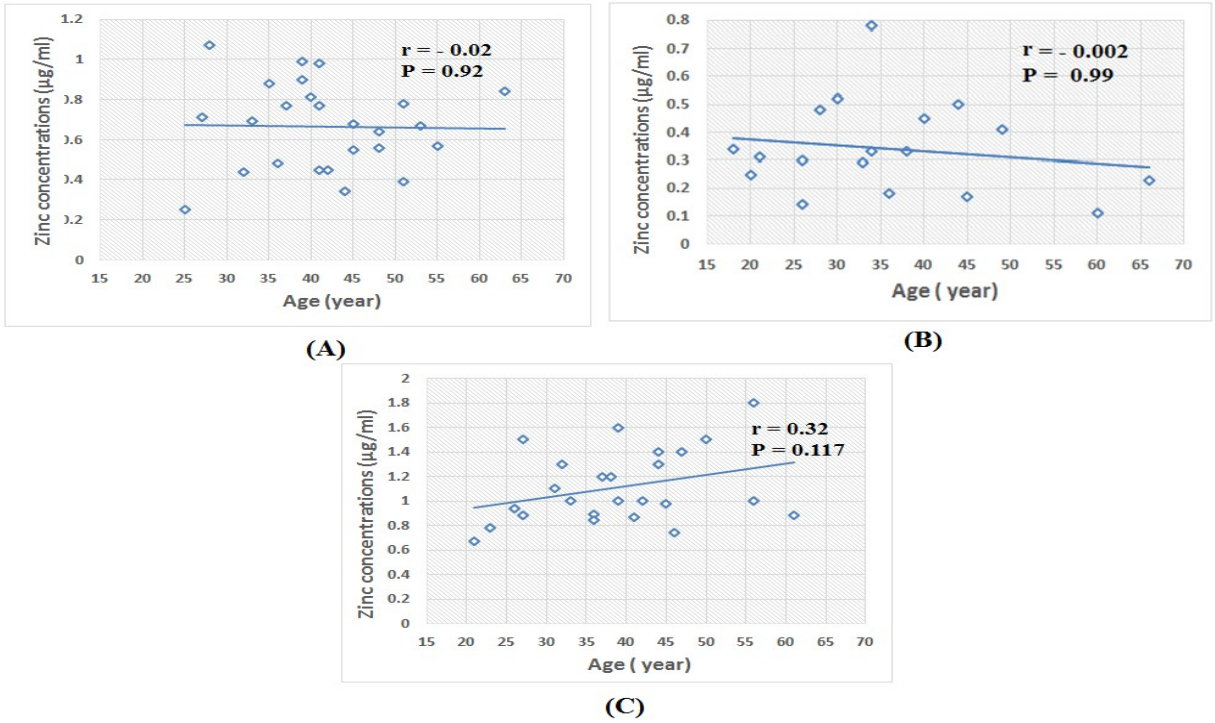

Fig. 6: Association between age and salivary zinc contents in different groups (n=25 each). (A) non- SARS-CoV-2 subjects (n=25), (B) SARS-CoV-2 subjects (n=25) and (C) zinc -SARS-CoV-2 treated subjects (n=25).

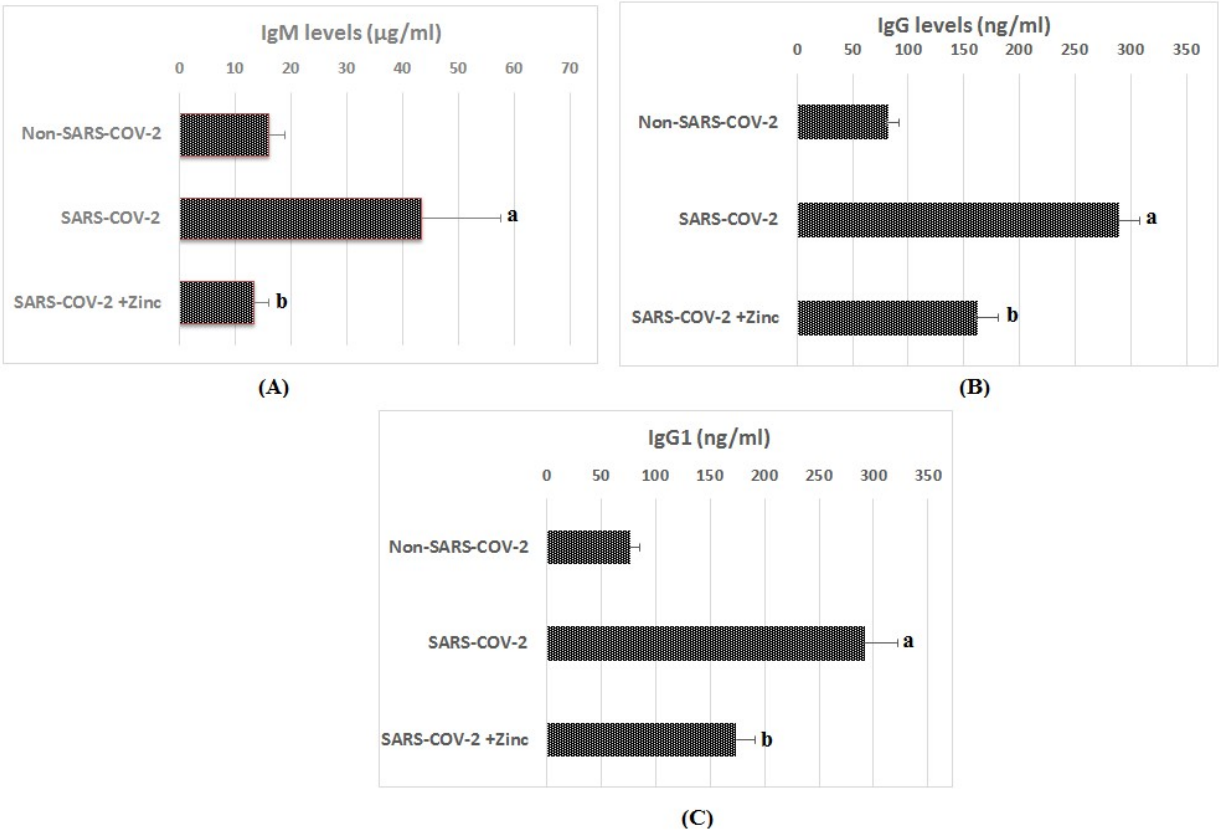

Fig. 7: Levels of SARS-CoV-2 spike-specific antibodies in studied groups. (A) IgM, (B) IgG and (C) IgG1. Results are calculated as mean ±SD. [a]P≤0.001 with relation to non- SARS-CoV-2 subjects, [b]P≤0.001 versus SARS-CoV-2 subjects.

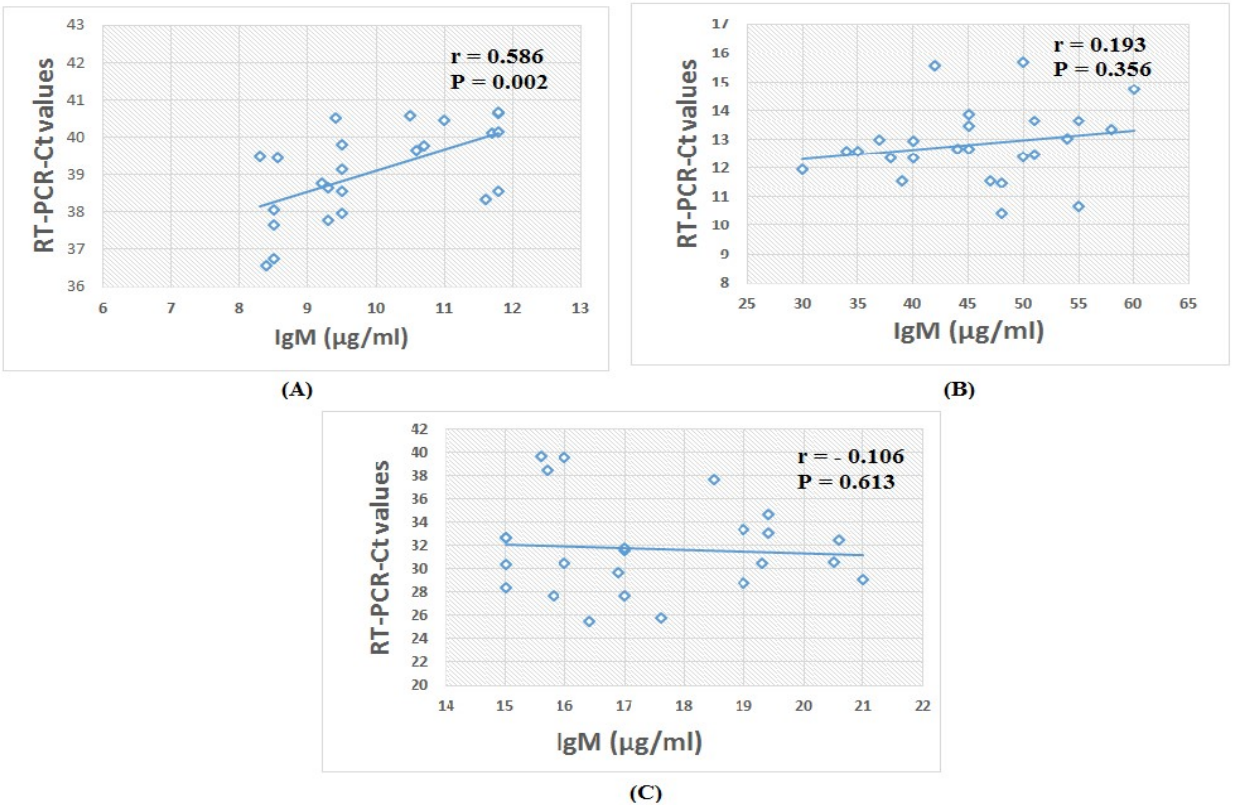

Fig. 8: Relationship between serum IgM levels and RT-PCR-Ct values in studied groups. (A) non- SARS-CoV-2 subjects (n=25), (B) SARS-CoV-2 subjects (n=25) and (C) zinc -SARS-CoV-2 treated subjects (n=25).

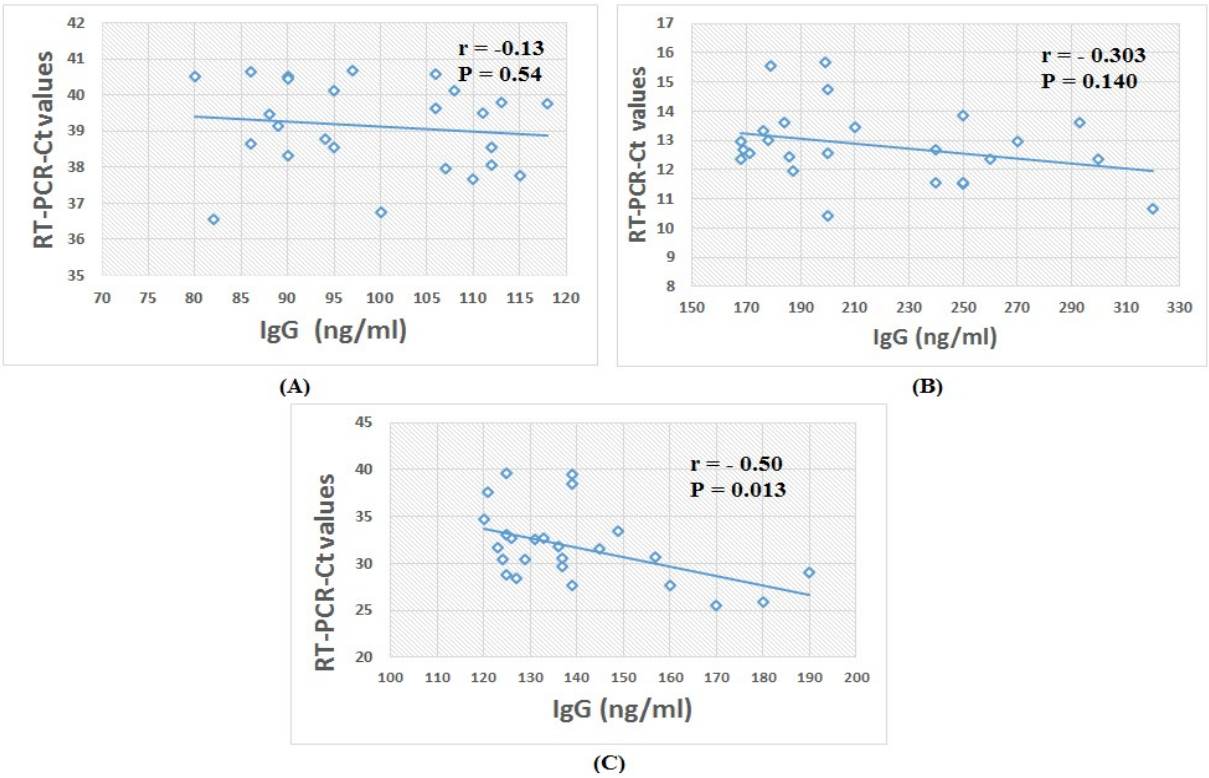

Fig. 9: Relationship between serum IgG levels and RT-PCR-Ct values in studied t groups. (A) non- SARS-CoV-2 subjects (n=25), (B) SARS-CoV-2 subjects (n=25) and (C) zinc -SARS-CoV-2 treated subjects (n=25).

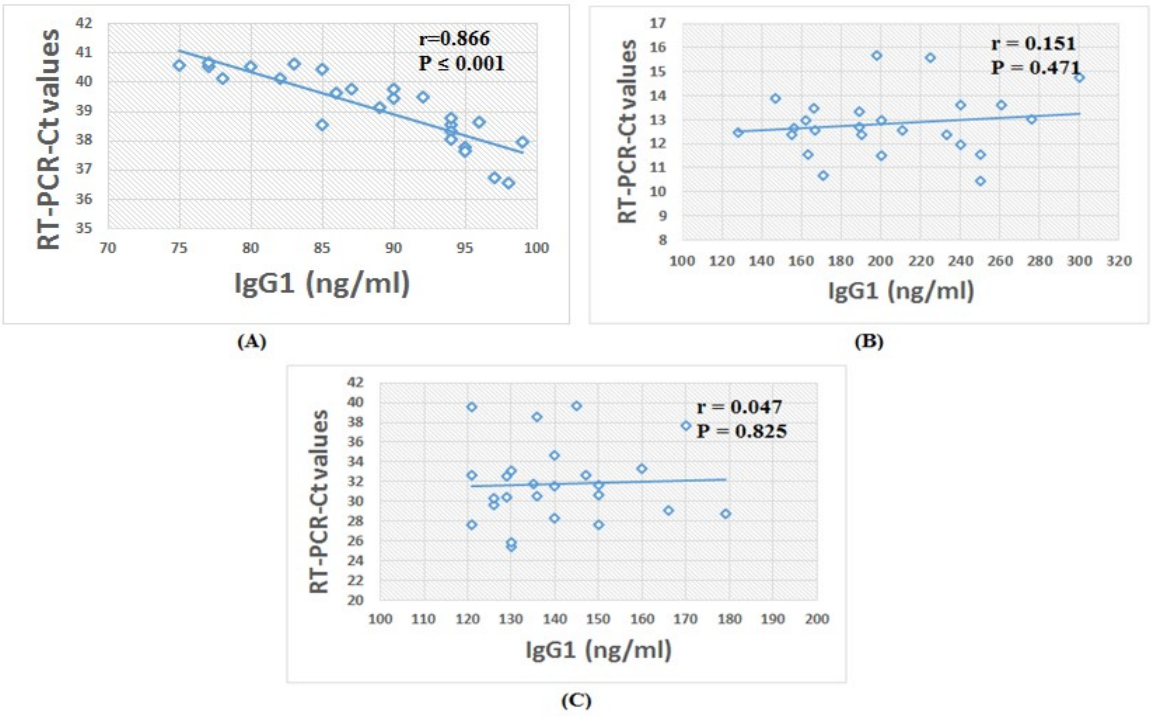

Fig. 10: Relationship between serum IgG1 levels and RT-PCR-Ct values in studied groups. A) non- SARS-CoV-2 subjects (n=25), (B) SARS-CoV-2 subjects (n=25) and (C) zinc -SARS-CoV-2 treated subjects (n=25).

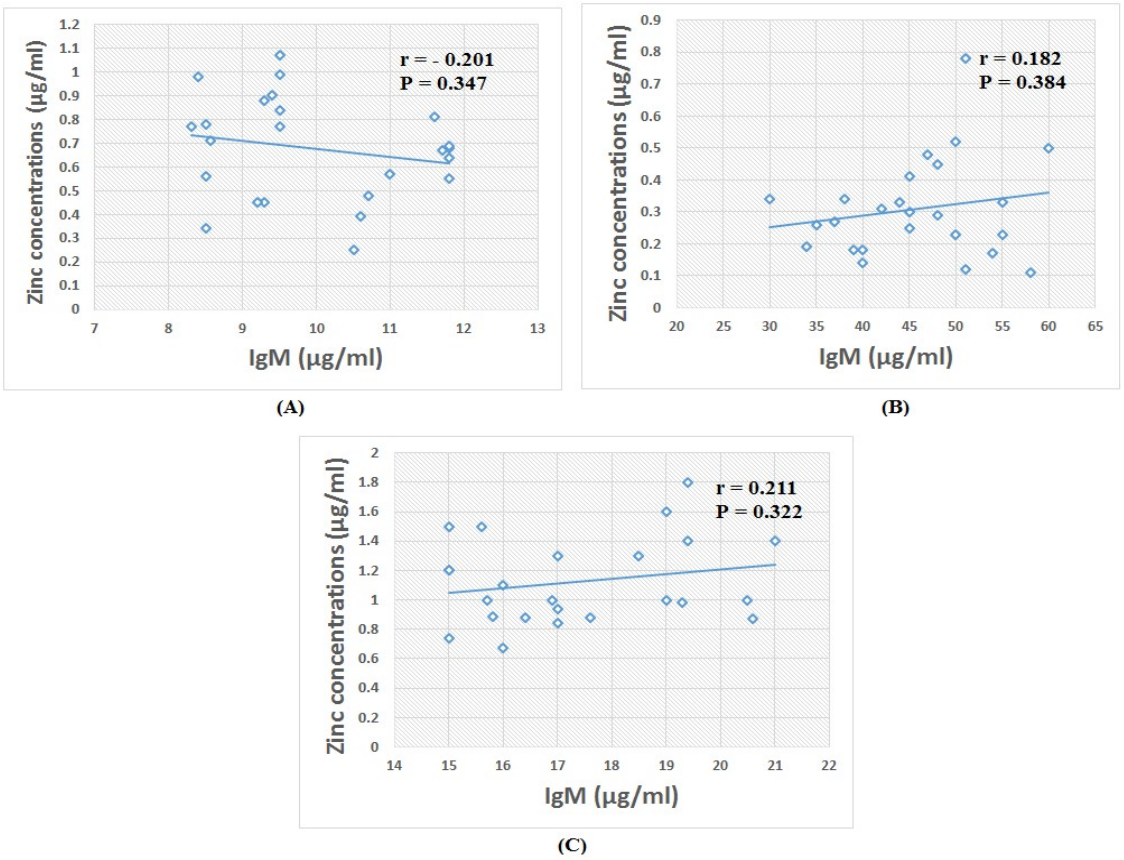

Fig. 11: Relationship between serum IgM levels and salivary zinc concentrations in studied groups. (A) non- SARS-CoV-2 subjects (n=25), (B) SARS-CoV-2 subjects (n=25) and (C) zinc -SARS-CoV-2 treated subjects (n=25).

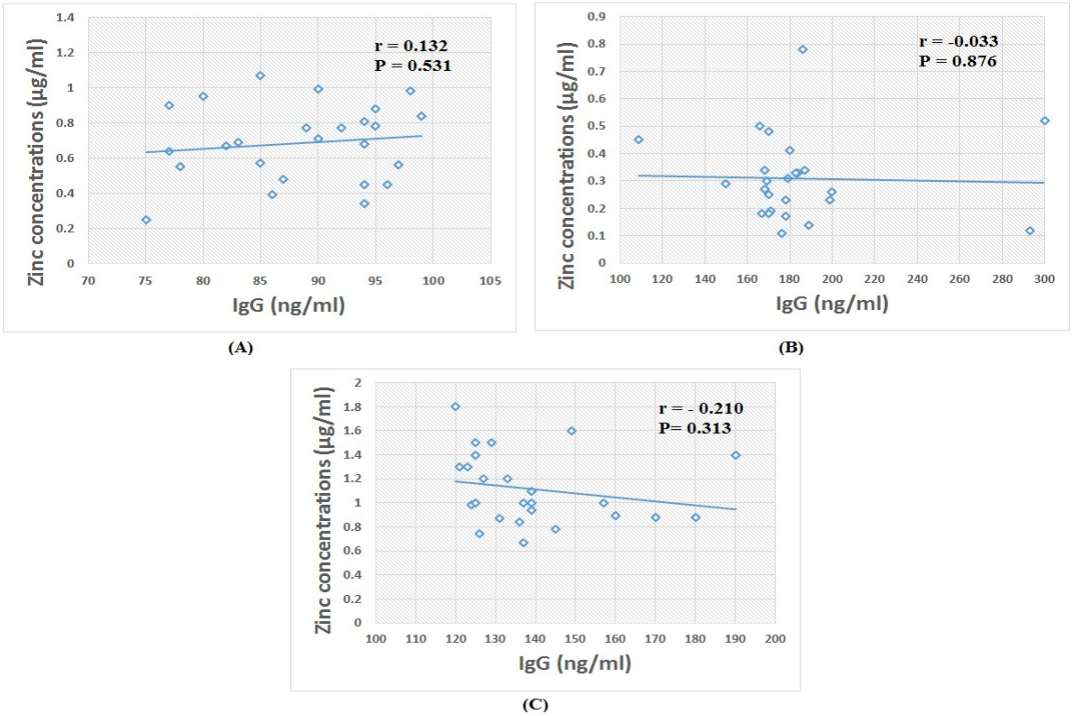

Fig. 12: Relationship between serum IgG levels and salivary zinc concentrations in studied groups. (A) non- SARS-CoV-2 subjects (n=25), (B) SARS-CoV-2 subjects, (C) zinc -SARS-CoV-2 treated subjects (n=25).

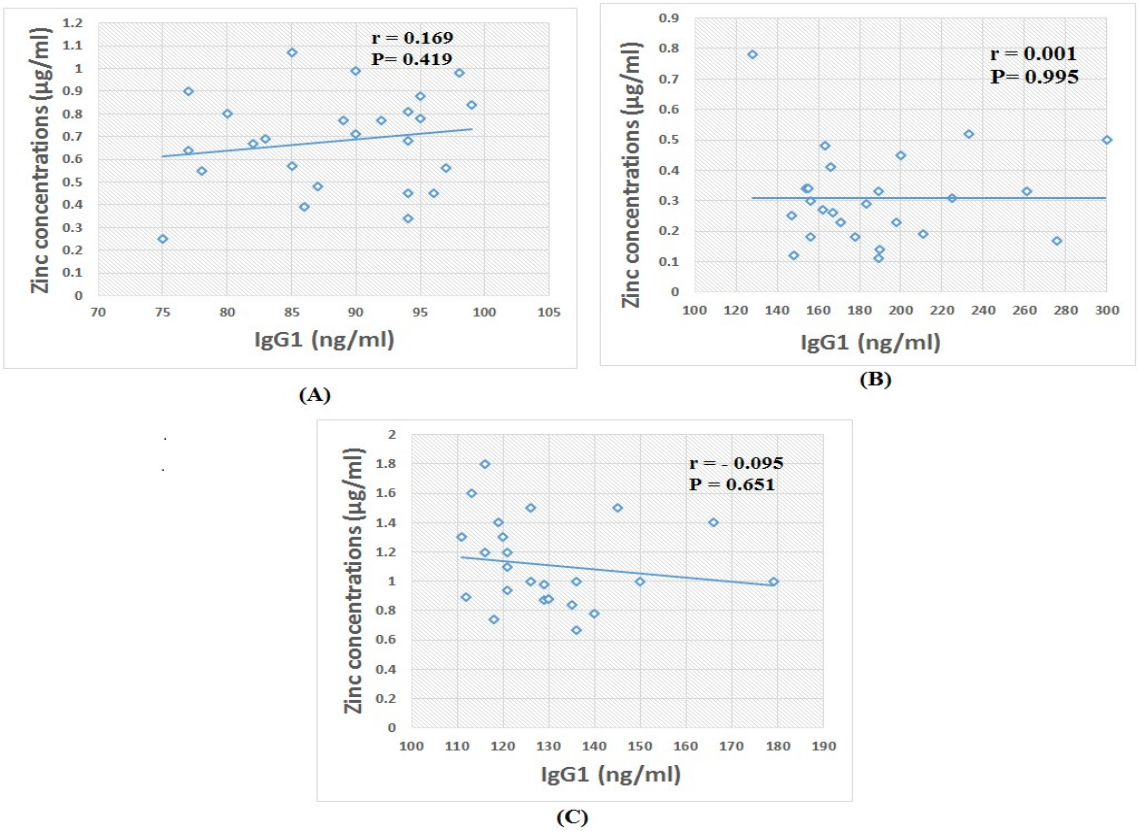

Fig. 13: Relationship between serum IgG1 levels and salivary zinc concentrations in studied groups. (A) non- SARS-CoV-2 subjects (n=25), (B) SARS-CoV-2 subjects (n=25) and (C) zinc -SARS-CoV-2 treated subjects (n=25).

IgG showed non-significant positive correlation with salivary zinc concentrations in healthy individuals (r= 0132, P=0.531, fig. 12A), however inversely weak correlation was identified between this antibody and salivary zinc concentrations in SARS-CoV-2 subjects (r= -0.033, P=0.876, fig. 12B) and SARS-CoV-2 subjects treated with zinc (r=-0.210, P=0.313, fig. 12C). Weak positive correlation was observed between IgG1 and salivary zinc concentrations in healthy individuals (r=0.169, P=0.419, fig. 13A) and SARS-CoV-2 subjects (r=0.001, P=0.995, fig. 13B), while there was a weak inverse relationship between this antibody and salivary zinc concentrations in SARS-CoV-2 subjects treated with zinc (r = -0.095, P = 0.651, fig. 13C).

DISCUSSION

It is generally recognized that the susceptibility to viral infection is greatly influenced by the host's nutritional status. Several investigations proposed that nutritionally deficient individuals are e more susceptible to viral invasion or have severe infections (Beck and Matthews, 2000; Gombart *et al.*, 2020; Balboni *et al.*, 2022).

This research work aimed to explore the link between SARS-CoV-2 viral burden (as shown by RT-PCR-Ct values) and salivary zinc concentrations as well as the levels of serum specific antibodies (IgM, IgG and IgG1) against viral spike protein in infected subjects. In addition, the study demonstrated the influence of zinc therapy on alleviating RT-PCR-Ct values and antibody levels in subjects with viral infections.

75 participants were included in this investigation, classified as 25 healthy individuals (non-SARS-CoV-2), 25 SARS-CoV-2 infected subjects and 25 infected ones treated with zinc.

SARS-CoV-2 viral burden has been evaluated by the cycle threshold value (Ct-value) of a real-time reverse transcription polymerase chain reaction (RT-PCR) assay, which can assist in diagnosing individuals at high risk of developing a serious illness (Zou *et al.*, 2020). The present result demonstrated low RT-PCR-Ct values (< 30) in SARS-CoV-2 diagnosed subjects versus healthy participants. The drop in RT-PCR-Ct values revealed high levels of viral RNA (high viral burden). This result is consistent with another study that reported depletion of RT-PCR-Ct values during the viral invasion outbreak, proposing that the collective RT-PCR-Ct values may be considered an indicator of the whole viral burden in the affected area at the population scale (Quiroga *et al.*, 2021). According to earlier research, a high viral burden may have a fundamental role in the riskiness of viral infections and be linked to higher mortality rates (Pujadas *et al.*, 2020). The current study also revealed salivary zinc levels were markedly lower in virally infected individuals versus healthy individuals.. This finding is parallel with former investigations declaring hypozincemia in infected patients (Jothimani *et al.*, 2020; Heller *et al.*, 2021; Xu *et al.*, 2022), highlighting the significance of preserving a healthy zinc balance. Pearson's correlation presented in the current study revealed a significant positive relationship between salivary zinc level values in SARS-CoV-2 subjects (r=0.453, P=0.023), indicating that subjects with low salivary zinc have low RT-PCR-Ct values (high viral burden). From this correlation, it can be suggested that the high viral burden in these individuals may correlate to zinc deficiency, which may aggravate the severity of the viral infection. Zinc deficiency in virally infected individuals may cause immune dysfunction, leading to increased vulnerability to viral invasion (Helge and Lothar, 2003). The World Health Organisation (WHO, 2003) reported a strong connection between zinc insufficiency and vulnerability to severe viral infection worldwide, recommending the potential beneficial influence of zinc therapy. Also, Yasui *et al*. (2020) demonstrated a close connection between hypozincemia and the seriousness of SARS-CoV-2 infection, proposing that serum zinc levels can be considered a sign of a severe ailment in SARS-CoV-2 cases. Moreover, Beck and Levander (2000) reported that zinc deficiency could lead to viral mutation in a new, more virulent strain. In this context, zinc supplementation has been performed in the current study, aiming to reduce the poor outcome resulting from the severity of SARS-CoV-2 invasion. The data revealed that zinc supplementation (50mg) twice daily for 30 successive days, greatly improved the clinical outcomes indicated by increasing the clinical RT-PCR-Ct values and salivary zinc levels versus untreated infected individuals. A significant positive linkage between salivary zinc levels and RT-PCR-Ct values was noticed in the present study in virally infected individuals after treatment with zinc (r=0.521, P=0.008), proposing the vital role of zinc in viral clearance in patients with severe infection. Previous studies have demonstrated that zinc treatment can help in virus eradication via its direct effect on the virus and/or by boosting the immunity of patients against SARS-CoV-2 invasion (Prasad *et al.,* 2022). Earlier publications declared that zinc has an antiviral useful impact via suppressing the viral RNA polymerase and decreasing the action of the viral receptor, namely angiotensin-converting enzyme-2 (Velthuis *et al.*, 2010; Warnes *et al.*, 2015, Wessels *et al.*, 2020). Moreover, zinc can interfere with viral penetration into the cell via stabilization of the cell membrane (Pasternak, 1987). Shakoor *et al*. (2021) hypothesized that zinc may inhibit viral binding, uncoating and replication, which aid in the decline of the viral burden in severe SARS-CoV-2 infection cases. Furthermore, zinc can enhance antiviral immunity via increasing the generation of interferon cytokine and boosting its antiviral action (Afjadi *et al.*, 2021). Clinical studies have proposed that zinc therapy may shorten the symptom duration, promote

the phagocytic activity of lymphocytes and enhance the response to immunotherapy in many viral invasion (Gammoh and Rink, 2017).

On studying the impact of gender on salivary zinc concentrations, the results showed that the lack of zinc was markedly more prevalent in females than males within each studied group. This observation is in line with a clinical study that found that males have a considerable higher plasma zinc concentration than females (Alqabbani and AlBadr, 2020); however, another investigation stated that no appreciable variations in zinc levels have been found between males and females (Romero *et al.*, 2002). Regarding the impact of age on salivary zinc, the data showed non-significant variations in the salivary zinc concentrations between the different age classes (20–40 and 40–60) within each studied group. Pearson's correlation showed a non-significant association between age and salivary zinc concentrations within each studied group.

Antibody-mediated humoral immune reactions are one of the crucial elements for developing immunity and avoiding re-infection. The current research estimates the serum contents of specific antibodies (IgM, IgG and IgG1) versus SARS-CoV-2 spike antigen in infected subjects in comparison to healthy individuals. The results revealed that SARS-CoV-2 subjects with a high viral burden had pronouncedly higher concentrations of IgM, IgG and IgG1 versus healthy uninfected individuals. These results may confirm that immune responses are generated following SARS-CoV-2 infection, suggesting that infected subjects could rapidly gain immunity against viral invasion. Deshpande *et al*. (2021) declared that the production of spike specific antibodies in the sera of patients may decrease their mortality rate, confirming the notion that the humoral immune response has a defensive effect during the course of viral invasion that may serve to avoid re-infection over time. Also, the same authors added that the severe infection was related to more vigorous immune responses and higher IgG levels (Deshpande *et al.*, 2021). The present statistical analysis showed non-significant correlation between viral burden and IgM, IgG and IgG1 in group of viral infected patients. The significant depletion in antibody levels in virally infected individuals after treatment with zinc versus untreated SARS-CoV-2 subjects may confirm the capability of zinc to reduce the viral burden presented in the current study. A strong inverse association was observed between IgG levels and RT-PCR-Ct values in SARS-CoV-2 patients treated with zinc ($r = -0.50$, $P \leq 0.013$), indicating that patients with lower viral burden have lower levels of IgG. This result may confirm the ability of zinc to combat SARS-CoV-2 viral invasion via its direct effect on the virus, as previously mentioned (Pasternak, 1987; Velthuis *et al.*, 2010; Wessels *et al.*, 2020), thus downregulating the systemic humoral immunological response. Concerning the relation of salivary zinc levels with serum antibody levels (IgM, IgG, and IgG1), the result showed that no strong correlation has been found between the concentrations of the used micronutrient and the serum antibody levels.

CONCLUSION

The current results showed that SARS-CoV-2 infected subjects with a high viral load have a deficient salivary zinc level. SARS-CoV-2 individuals treated with zinc (50mg) twice daily for 30 days showed a lower viral load than untreated ones, proposing the important role of zinc supplementation in viral eradication in subjects with severe infections. Also, our study revealed higher production of specific IgM, IgG and IgG1 antibodies against viral spike antigen in virally infected individuals versus healthy ones. Zinc therapy showed lower antibody levels in virally infected individuals versus untreated infected ones. The strong negative correlation between IgG levels and RT-PCR-Ct values in SARS-CoV-2 subjects treated with zinc may confirm that zinc supplementation has a direct impact on viral clearance and may be considered a useful therapeutic strategy against the severity of viral infection.

REFERENCES

Afjadi M N, Karami H, Goudarzi K, Alipourfard I and Bahreini E (2021). The effect of vitamin D, magnesium and zinc supplements on interferon signaling athways and their relationship to control SARS-CoV-2 infection. *Clin. Mol. Allergy*, **19**(1): 1e10.

Almasaud AS, Chalabi J, Arfaj AA, Qarni AA, Alkroud A, Nagoor Z, Akhtar S and Iqbal J (2023). Association of serum zinc and infammatory markers with the severity of Covid-19 infection in adult patients. *Nutrients*, **15**(2): 340.

Alqabbani HM and AlBadr NA (2020). Zinc status (intake and level) of healthy elderly individuals in Riyadh and its relationship to physical health and cognitive impairment. *Clin. Nutr. Exp*., **29**: 10e17.

Balboni E, Zagnoli F, Filippini T, Fairweather-Tait SJ and Vinceti M (2022). Zinc and selenium supplementation in COVID-19 prevention and treatment: a systematic review of the experimental studies. *J. Trace Elem. Med. Biol*., **71**: 126956.

Bastos ML, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP, Johnston JC, Lan Z, Law S, Lean EM, Trajman A, Menzies D, Benedetti A and Khan FA (2020). Diagnostic accuracy of serological tests for covid-19 systematic review and meta-analysis. *BMJ*, **370**: m2516.

Beck MA and Matthews CC (2000). Micronutrients and host resistance to viral infection. *Proc. Nutr. Soc*., **59**(4): 581-585.

Beck MA and Levander OA (2000). Host nutritional status and its effect on a viral pathogen. *J. Infect. Dis*., **182**(Suppl 1): S93-96.

Bonaventura P, Benedetti G, Albar`ede F and Miossec P (2015). Zinc and its role in immunity and inflammation. *Autoimmun Rev*., **14**(4): 277-285.

Chernecky C and Berger B (2008). Laboratory tests and diagnostic procedures 5th ed. St Louis, MO, Saunders.

Deshpande G, Kaduskar O, Deshpande K, Bhatt V, Yadav P, Gurav Y, Potdar V, Khutwad K, Vidhate S, Salunke A, Patil C, Shingade S, Jarande K, Tilekar B, Salvi P, Patsuthe S, Dange V, Kumar S, Gurav S, Chate S, Abraham P and Gajanan S (2021). Longitudinal clinico-serological analysis of anti-nucleocapsid and anti-receptor binding domain of spike protein antibodies against SARS-CoV-2. *Int. J. Infect. Dis*., **112**: 103-110.

Dhama K, Dhawan M, Tiwari R, Emran TB, Mitra S, Rabaan AA, Alhumaid S, Alawi ZA and Al Mutair A(2022). COVID-19 intranasal vaccines: Current progress, advantages, prospects and challenges, *Hum. Vaccines Immunother*, **18**(5): 1-11.

Finzi E (2020). Treatment of SARS-CoV-2 with high dose oral zinc salts: a report on four patients, *Int. J. Infect. Dis*., **99**: 307-309.

Gammoh NZ and Rink L (2017). Zinc in infection and inflammation. *Nutrients*, **9**(6): 624.

Gombart AF, Pierre A and Maggini S (2020). A review of micronutrients and the immune system-working in harmony to reduce the risk of infection. *Nutrients*, **12**(1): 236.

Guan W, Yue H, Bai X, Wang J, Yu Q, Liu Pu J, Wang X, Hu J, Xu D, Li X, Kang N, Li L, Lu W, Feng T, Ding L, Li X and Qi X (2020). Clinical characteristics of coronavirus disease 2019 in China. *N. Eng. J. Med*., **382**(18): 1708-1720.

Hallmans G (1978). Absorption of topically applied zinc and changes in zinc metabolism during wound healing. An experimental and clinical investigation. *Acta Derm. Venereo*., **58 (**80): 1-36.

Helge IK and Lothar R (2003). Zinc-altered immune function. *J. Nutr*., **133**(5 Suppl 1): 1452S-6S.

Heller RA, Sun Q, Hackler J, Seelig J, Seibert L, Cherkezov A, Minich WB, Seemann P, Diegmann J, Pilz M, Bachmann M , Ranjbar A, Moghaddam A and Schomburg L (2021). Prediction of survival odds in COVID-19 by zinc, age and selenoprotein P as composite biomarker. *Redox Biol*., **38**: 101764.

Ingberg E, Ahlstrand E, Cajander P, Sundqvist ELM, Wegener M, Lidén M and Cajander S (2022). RT-PCR cycle threshold value in combination with visual scoring of chest computed tomography at hospital admission predicts outcome in COVID-19. *Infect. Dis*., **54**(6): 431-440.

Ishay Y, Kessler A, Schwarts A and Ilan Y (2020). Antibody response to SARS-Co-V-2, diagnostic and therapeutic implications. *Hepatol. Commun*., **4**(12): 1731-1743.

Jothimani D, Kailasam E, Danielraj S, Nallathambi B, Ramachandran H, Sekar P, Manoharan S, Ramani V, Narasimhan G, Kaliamoorthy I and Rela M (2020). COVID-19: poor outcomes in patients with zinc deficiency. *Int. J. Infect. Dis*., **100**: 343-349.

Kontou PI, Braliou GG, Dimou NL, Nikolopoulos G and Bagos PG (2020). Antibody tests in detecting SARS-CoV-2 infection: A meta-analysis. *Diagnostics* **10**(5): 319-333.

Li D and Li J (2020). Immunologic Testing for SARS-CoV-2 Infection from the Antigen Perspective. *J. Clin. Microbiol*., **59** Antibody tests in detecting SARS-CoV-2 infection: A meta-analysis e02160.

Pal A, Squitti R, Picozza M, Pawar A, Rongioletti M, Dutta AK, Sahoo S. Goswami, K. Sharma P and Prasad R (2021). Zinc and COVID-19: Basis of current clinical trials. *Biol. Trace Elem. Res*., **199**(8): 2882-2892.

Pasternak CA (1987). A novel form of host defence: Membrane protection by Ca^{2+} and Zn^{2+}. *Biosci. Rep*., **7**(2): 81-91.

Prasad AS (2008). Zinc in human health: effect of zinc on immune cells. *Mol. Med*., **14**(5-6): 353-357.

Prasad AS, Malysa A, Bepler G, Fribley A and Bao B (2022). The Mechanisms of zinc action as a potent anti-viral agent: The clinical therapeutic implication in COVID-19. *Antioxidants*, **11**(10): 1862.

Pujadas E, Chaudhry F, McBride R, Richter F, Zhao S, Wajnberg A, Nadkarni G, Glicksberg BS, Houldsworth J and Cardo CC (2020). SARS-CoV-2 viral load predicts COVID-19 mortality. *Lancet Respir. Med*., **8**(9): e70.

Quiroga SA, Hern´andez C, Casta˜neda S, Paula Jimenez P, Vega L, Gomez M, Martinez D, Ballesteros N, Muñoz M, Cifuentes C, Sierra N, Flórez C, Paniz-Mondolfi A and Ramírez JD (2021). Contrasting SARS-CoV-2 RNA copies and clinical symptoms in a large cohort of Colombian patients during the first wave of the COVID-19 pandemic. *Ann. Clin. Microbiol. Antimicrob*., **20**: 39.

Rahman MT and Idid SZ (2021). Can Zn be a critical element in COVID-19 treatment? *Biol. Trace Elem. Res*., **199**(2): 550-558.

Romero CD, Sanchez PH, Blanco FL, Rodríguez ER and Majem LS (2002). Serum copper and zinc concentrations in a representative sample of the Canarian population. *J. Trace Elem. Med. Biol*., **16(**2): 75e81.

Shakoor H, Feehan J, A.S. Dhaheri AS, Ali HI, Platat C, Ismail LC, Apostolopoulos V and Stojanovska L (2021). Immune-boosting role of vitamins D, C, E, zinc, selenium and omega-3 fatty acids: could they help against COVID-19? *Maturitas*, **143**(1): 1-9.

Te Velthuis AJ, van den Worm SH, Sims AC, Baric RS, Snijder EJ and van Hemert MJ (2010). Zn^{2+} inhibits

coronavirus and arterivirus RNA polymerase activity *in vitro* and zinc ionophores block the replication of these viruses in cell culture. *PLoS Pathogens*, **6**(11): e1001176.

Theel ES, Slev P, Wheeler S, Couturier MR, Wong SJ and Kadkhoda K (2020). The role of antibody testing for SARS-CoV-2: Is there one? *J. Clin. Microbiol*., **58**(8): e00797–20.

To KKW, Tsang OTY, Leung WS, Tam AR, Wu TC, Lung DC, Yip CCY, Cai JP, Chan JMC, Chik TSH, Lau DPL, Choi CYC, Chen LL, Chan WM, Chan KH, Ip JD, Ng ACK, Poon RWS, Luo CT, Cheng VCC, Chan JFW, Hung IF, Chen Z, Honglin Chen H and Yuen KY (2020) . Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect. Dis*., **20**(5): 565-574.

Walsh, K, Jordan K, Clyne B, Rohde D, Drummond L, Byrne P, Ahern, S, Carty PG, O'Brien K, O'Murchu E, O'Neill M, Smith SM, Ryan M and Harrington P (2020). SARS-CoV-2 detection, viral load and infectivity over the course of an infection. *J. Infect*., **81**(3): 357-371.

Warnes SL, Little ZR and Keevil CW (2015). Human coronavirus 229E remains infectious on common touch surface materials. *mBio*., **6**(6): e01697- 15.

Wessels I, Rolles B and Rink L (2020). The potential impact of Zinc supplementation on COVID-19 pathogenesis, *Front. Immunol*., **11**: 1712.

WHO (2003). The world health report 2002, *Midwifery* **19**(1): 72-3.

Xu W, Liu Y, Zou X, Luo H, Wu W, Xia J, Chan MT, Fang S, Shu Y, Wu WK and Zhang L (2022). Hypozincemia in Covid-19 patients correlates with stronger antibody response. *Front Immunol*., **12**: 785599.

Yasui Y, Yasui H, Suzuki K, Saitou T, Yamamoto Y, Ishizaka T, Nishida K, Yoshihara S, Gohma I and Ogawa Y (2020). Analysis of the predictive factors for a critical illness of COVID-19 during treatment-relationship between serum zinc level and critical illness of COVID-19. *Int. J. Infect. Dis*., **100**: 230-236.

Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, Yu J Kang M, Song Y, Xia J, Guo Q, Song T, He J, Yen HL, Peiris M and Wu J (2020). SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N. Engl. J. Med*., **382**: 1177-1179.