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Research Article

Effects of SARS-CoV-2 on DNA Damage Response Proteins Chk1 and P53: An *in vitro* Study

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Abstract

Background: Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has had a global impact on millions of people's lives. Deteriorations in cellular activities induced by this lethal virus are not yet completely understood, and so its long-term consequences are unknown. There is increasing evidence that SARS-CoV-2 and its vaccinations may have a deleterious influence on DNA damage response (DDR)-associated proteins. ***Objective***: To investigate the status of DNA integrity in COVID-19-recovered patients and post-recovered vaccinated individuals. ***Methods***: Blood samples were taken from 88 participants who completed questionnaires and conducted face-to-face interviews. The samples were classified into four categories based on the subjects' PCR and vaccination status: PCR negative/not vaccinated, PCR positive/not vaccinated, PCR negative/vaccinated, and PCR positive/vaccinated. ELISA kits were used to determine the expression levels of TP53BP1, Chk1, and SARS-CoV-2 IgG proteins. ***Results***: SARS-CoV-2 did not significantly reduce Chk1 expression, but it did have a significant negative influence on TP53BP1 expression when compared to the first group. Infection with SARS-CoV-2 and its vaccination resulted in increased Chk1 and IgG levels, as well as a significant increase in TP53BP1 expression compared to the second group. ***Conclusions***: Both SARS-CoV-2 infection and the anti-SARS-CoV-2 vaccine may have a deleterious influence on DDR-associated proteins in vitro. Post-infection immunization may boost viral protection. While some studies imply that DDR effects are reversible, more research is needed to corroborate these assertions.

Keywords: Chk1, COVID-19, DNA damage, IgG, p53.

آثار SARS-CoV-2 على بروتينات الاستجابة لتلف الحمض النووي Chk1 و P53: دراسة في المختبر

الخلاصة

الخلفية: كان لفيروس كورونا -2 (SARS-CoV-2) تأثير عالمي على حياة ملايين الأشخاص. إن التدهور في الأنشطة الخلوية الناجم عن هذا الفيروس الفتاك غير مفهوم تماما بعد ، وبالتالي فإن عواقبه طويلة المدى غير معروفة. هناك أدلة متزايدة على أن SARS-CoV-2 ولقاحاته قد يكون لها تأثير ضار على البروتينات المرتبطة باستجابة تلف الحمض النووي (DDR). **الهدف**: التحقيق في حالة سلامة الحمض النووي لدى المرضى المتعافين من COVID-19 والأفراد الذين تم تطعيمهم بعد التعافي. **الطرائق**: تم أخذ عينات الدم من 88 مشاركا أكملوا الاستبيانات وأجروا مقابلات وجها لوجه. تم تصنيف العينات إلى أربع فئات بناء على تفاعل البوليميراز المتسلسل وحالة التطعيم الخاصة بالأشخاص: تفاعل البوليميراز المتسلسل سلبي/غير ملقح، إيجابي تفاعل البوليميراز المتسلسل/غير ملقح ، تفاعل البوليميراز المتسلسل سلبي/ملقح، وإيجابي تفاعل البوليميراز المتسلسل/ملقح. تم استخدام مجموعات ELISA لتحديد مستويات التعبير عن بروتينات TP53BP1 و Chk1 و SARS-CoV-2 IgG. **النتائج**: لم يقلل SARS-CoV-2 بشكل كبير من تعبير Chk1، ولكن كان له تأثير سلبي كبير على التعبير TP53BP1 عند مقارنته بالمجموعة الأولى. أدت الإصابة ب SARS-CoV-2 والتطعيم إلى زيادة مستويات Chk1 و IgG ، فضلا عن زيادة كبيرة في التعبير TP53BP1 مقارنة بالمجموعة الثانية. **الاستنتاجات**: قد يكون لكل من عدوى SARS-CoV-2 واللقاح المضاد ل SARS-CoV-2 تأثير ضار على البروتينات المرتبطة ب DDR في المختبر. علاوة على ذلك ، تشير النتائج إلى أن التمنيع بعد العدوى قد يعزز الحماية الفيروسية. في حين أن بعض الدراسات تشير إلى أن تأثيرات نزع السلاح والتسريح وإعادة الإدماج قابلة للعكس، إلا أن هناك حاجة إلى مزيد من البحث لتأكيد هذه التأكيدات.

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INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is a highly contagious pathogenic human coronavirus isolated first from the respiratory epithelia of pneumonia patients in Wuhan, China [1]. SARS-CoV-2 causes coronavirus disease 19 (COVID-19), which quickly became a serious global health problem [2]. As the virus spreads globally, it faces numerous obstacles and defenses within the

host's immune system. The speed and pattern of the SARS-CoV-2 infection worry researchers about the potential risks that it may pose to humanity as it changes and evolves over time due to mutations [3,4]. Given the likelihood of the SARS-CoV-2 virus becoming endemic in humans, it is crucial to further investigate the long-term effects of the initial infection in COVID-19-recovered patients. Viruses exploit the host's cellular machinery for replication and spread, often involving various cellular processes, including the DDR [5]. Several studies have described the potential for DNA damage and the consequences of DDR associated with SARS-CoV-2 viral infection in the human host. Individuals who have recovered from COVID-19 may still experience persistent symptoms and a general feeling of malaise well after the infection has subsided [6]. This could indicate that SARS-CoV-2 infection has a significant impact on the host's DNA repair system. Previous studies have described several mechanisms by which host-induced DNA damage and DDR are modulated by SARS-CoV-2. This could involve interactions with DNA polymerase that lead to DNA damage, DNA replication fork stress, phosphorylation of H2AX histones, and a stop in the cell cycle in the S phase. Scientists have also found that SARS-CoV-2 raises the amounts of ataxia telangiectasia and Rad3-related protein (ATR) by increasing transcription, which then supports phosphorylation checkpoint kinase 1 (Chk1) and H2AX [7,8]. Furthermore, other studies have found a possible association between SARS-CoV-2 infection and p53 downregulation or loss of function [9,10]. Different vaccines have been developed for immunization against this virus, some of which (e.g., Pfizer from BioNTech) have been successful [11]. According to Ebrahim *et al*. (2022), vaccination of individuals with prior SARS-CoV-2 infection with a single dose generates more protection in comparison to SARS-CoV-2-naive individuals [12]. Flor *et al*. (2023) also demonstrated in their study that post-vaccination infection increases immunization against the virus [13]. This may be significant from an immunization point of view, but assessing the long-term effects of deploying the vaccine in post-recovered patients is not well understood and requires further evaluation. It is also important to realize that most of the publications relate to the possible biological impacts of SARS-CoV-2 through *in vitro* studies; accordingly, *in vivo* investigations are a necessity to uncover the long-term effects. In this study, we aimed to learn more about the status of DNA integrity among COVID-19-recovered patients. We also tried to explore the potential impacts of anti-SARS-CoV-2 vaccines on DNA integrity in both previously infected and non-infected individuals. In the light of the outcomes of previous studies, we hypothesize a possible relationship between DNA integrity and infection with the SARS-CoV-2 virus. This work sought to expand our knowledge of how this virus might influence DDR proteins in recovered patients. Moreover, we were interested in studying how vaccination against the virus would affect the expression of DDR proteins in non-infected as well as previously infected individuals.

METHODS

Study design data and blood sample collection

This study was conducted in the Soran region of Erbil province and its neighboring districts—Rwanduz, Choman, and Khalifan—within the Kurdistan Region of Iraq. Data and blood samples were collected from November 2021 to the end of February 2022. Initially, questionnaires were randomly distributed among the local population, and face-to-face interviews were conducted with 2,368 individuals from diverse professional backgrounds, including students, teachers, healthcare workers, farmers, and personnel from the security and transportation sectors. Before beginning the project, the applied methodologies obtained approval from the Scientific and Ethical Committee of the Faculty of Education at Soran University. All participants gave their informed consent, either to fill out questionnaire forms or to donate blood samples. For choosing blood sample donors, we have included only those who conducted PCR tests to confirm their infection status and those who were living in the targeted area of the study. Meanwhile, we excluded participants who did not receive the two doses of the vaccine, those who suffered from chronic diseases, and those who lived near communication towers. Moreover, a significant number of participants declined to donate blood samples, either due to their busy schedules or their subsequent changing opinion. As a result, we were able to collect samples from only 88 of them who met our previously mentioned criteria after double-checking their profiles. We assigned four groups for subsequent evaluations after collecting blood samples: the control group (PCR negative/not vaccinated), the COVID-19 recovered patients' group (PCR positive/not vaccinated), the anti-SARS-CoV-2 vaccinated group (PCR negative/vaccinated), and the fourth group, the COVID-19 recovered patients who received the anti-SARS-CoV-2 vaccine (PCR positive/vaccinated). A trained phlebotomist collected peripheral blood samples from the donor's antecubital veins for phlebotomy. The blood samples were promptly placed into non-heparinized tubes and centrifuged at 5,000 rpm for 5 minutes. Once separated, the sera were transferred to 1.5 mL Eppendorf tubes and stored at -20 °C until analysis. All participants of this study visited the Covid-19 Testing Centre at Soran University to verify their infection status. The samples, either nasal or nasopharyngeal/oropharyngeal swabs, were processed according to the Centre for Disease Control and Prevention (CDC) guidelines. Basically, samples were kept at 4 ^{o}C until processed in a downstream procedure.

Qualitative detection of SARS-CoV-2 nucleic acid

The PowerChek® 2019-nCoV Real-time PCR Kit (KogeneBiotech, Republic of Korea) was used to detect SARS-CoV-2 nucleic acid in samples in accordance with the manufacturer's instructions, which are briefly described here. The QIAamp® DSP Viral RNA Mini Kit (QIAGEN, USA) was used to extract SARS-CoV-2 RNA from the patient's sample. To prepare the RT-PCR, the extracted RNA (4.5 µL) was added to a master mix (15.5 µL), for a total reaction volume of 20 µL. The PowerChek® 2019-nCoV Real-time PCR kit was used to quantitatively amplify the E and RdRP genes from viral RNA under the following conditions: 50℃ for 30 minutes, repeated 1 cycle, 95℃ for 10 minutes, repeated 1 cycle, 95℃ for 15 seconds, repeated 40 cycles, and fluorescence detection at 60℃ for 1 min. All the steps outlined above were carried out at the COVID-19 center in Soran, which is managed by the Kurdistan Regional Government's Ministry of Health in partnership with Soran University.

Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA kits used in this study were obtained from Sunlong Biotech Co. Ltd., China (www.sunlongbiotech.com) to quantify the expression levels of TP53BP1, Chk1, and SARS-CoV-2 IgG proteins. These kits included the human tumor protein p53 binding protein 1 (TP53BP1) [Ref. SL3730Hu], human checkpoint kinase 1 (Chk1) [Ref. SL3731Hu], and human anti-SARS-CoV-2 spike protein S1 IgG (S1-IgG) [Ref. SL3219Hu]. The assay procedures were conducted according to the manufacturer's instructions. Data from the ELISA plates were extracted using a microplate ELISA reader from BioTek (United States).

Statistical analysis

Data was given as mean ± standard error of the mean, and statistical analysis was performed using GraphPad Prism version 5.0 and SPSS version 19. We employed a one-way analysis of variance (ANOVA) to test for significance, followed by Duncan's multiple range comparison tests to compare means. We conducted Receiver Operator Characteristic (ROC) curve analysis using SPSS version 19 to determine correlations between groups. We regard *p*-values less than 0.05 as statistically significant.

RESULTS

To test the idea that SARS-CoV-2 infection causes DDR, we looked at the levels of Chk1 in serum samples from people in all three groups. According to the results of the ELISA test, neither getting SARS-CoV-2 nor getting the anti-SARS-CoV-2 vaccine significantly raises Chk1. Meanwhile, participants who had previously suffered from the SARS-CoV-2 infection and later received the vaccine experienced a slight increase in Chk1 levels (Figure 1A).

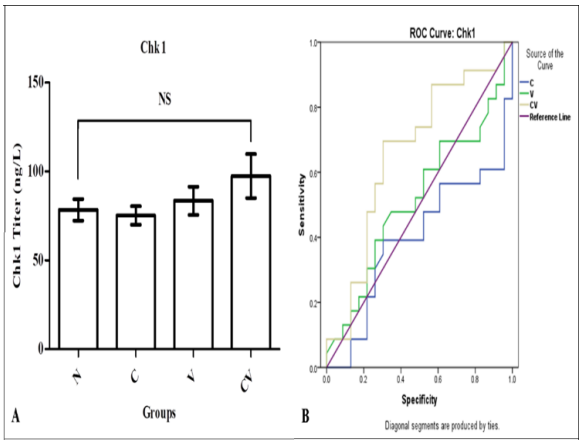

Figure 1: Comparison of Chk1 between the studied groups shows that SARS-CoV-2 infection did not trigger activation of downstream molecules of the ATR DNA damage response. A. One-way ANOVA and B. ROC data analysis were used to compare means and asterisks (*), if presented, represent significant differences at p<0.05. Abbreviations: C: PCR negative/not vaccinated, C: PCR positive/not vaccinated, V: PCR negative/vaccinated, CV: PCR positive/vaccinated.

The ROC data analysis did not show a significant positive correlation between the groups, as shown in Figure 1B. To determine whether SARS-CoV-2 infection or the anti-SARS-CoV-2 vaccine could modulate DNA damage repair by ceasing the recruitment of key cell cycle regulators, we measured the amount of DNA repair protein TP53BP1 in the sera of donors from all groups. The data analysis showed a slight decrease in TP53BP1 levels in SARS-CoV-2-infected participants compared to the control group and a significant decrease compared to participants who had previously been exposed to SARS-CoV-2 and received the vaccine (Figure 2A).

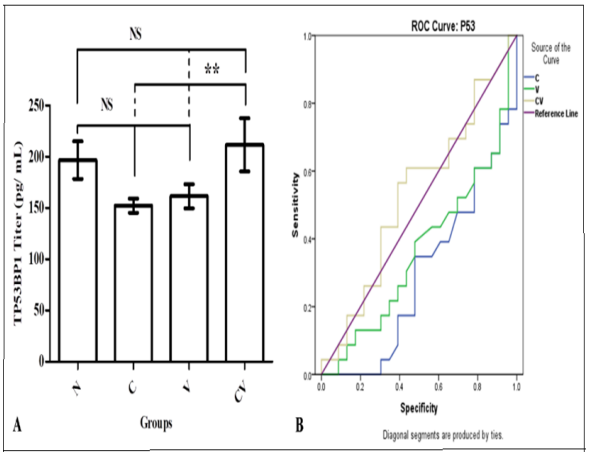

Figure 2: Comparison of the human tumour suppressor protein 53-binding proteins 1 (TP53BP1) DNA repair protein between the studied groups. A) One-way ANOVA and B) ROC data analysis was used to compare means and asterisks (*) represent significant differences at *p*<0.05. Abbreviations: C: PCR negative/not vaccinated, C: PCR positive/not vaccinated, V: PCR negative/vaccinated, CV: PCR positive/vaccinated.

The ROC data analysis did not show a significant positive correlation between the groups, as shown in Figure 2B. During a coronavirus infection, the

immune system first generates the IgM defense antibody, which is then followed by the formation of longer-lasting IgG-type-specific neutralizing antibodies. To learn more about this issue among our participants and find out if having been infected before and being vaccinated may increase the production of anti-SARS-CoV-2 IgG antibodies, we checked the levels of anti-SARS-CoV-2 IgG in the sera of participants from all groups (Figure 3). Our data indicates nonsignificant alterations in all groups and, at the same time, a marginal rise in the levels of anti-SARS-CoV-2 IgG in the fourth group (Figure 3A). The ROC data analysis did not reveal a statistically significant positive correlation between the groups as shown in Figure 3B.

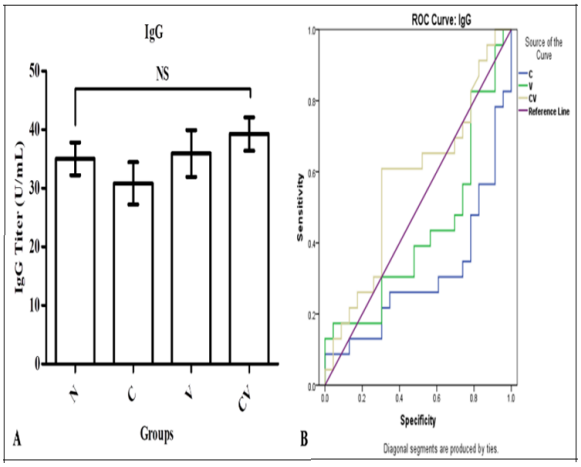

Figure 3: Comparison of serum levels of anti-SARS-CoV-2 IgG between studied groups. A) One-way ANOVA and B) ROC data analysis was used to compare means and asterisks (*), if presented, represent significant differences at $p<0.05$. Abbreviations: C: PCR negative/not vaccinated, C: PCR positive/not vaccinated, V: PCR negative/vaccinated, CV: PCR positive/vaccinated.

DISCUSSION

The worst viral pandemic to affect humanity since the Spanish flu, in the year 1918, was SARS-CoV-2. This pandemic encouraged researchers worldwide to conduct immediate studies to explore the biological features and rapid spread of this virus, as well as to study the relevant mutant coronaviruses that may cause deadly outbreaks. Most COVID-19 patients have shown a positive prognosis; however, significant health complications persist that may lead to high mortality rates among patients with critical conditions. Therefore, further investigation to understand viral pathogenicity and transmission routes is critical in the expectation of future outbreaks [14]. In order for viruses to replicate, they seize control of the cellular replication and expression machinery, creating viral progenies. While doing this, the foreign genomes of viruses leave traces of damage inside the infected cells with varying degrees of severity, including genome instability. Throughout their life cycles, many viruses can cause DNA damage and genome instability in host cells. The effects of SARS-CoVs on the DDR are not well understood, highlighting this as a significant area for further research [5,15]. Investigating the interactions between SARS-CoVs and the DDR not only provides valuable insights into how these viruses manipulate the host cell's DDR pathways but also enhances our understanding of their pathogenicity and strategies to inhibit viral replication. Our data shows that the SARS-CoV-2 infection did not turn on the Chk1 molecules that are part of the DDR pathway. Instead, we saw a tiny drop in Chk1 levels. We also noticed that Chk1 levels may be slightly higher in people who have already been infected and then been vaccinated. A previous study contradicted our findings by demonstrating that SARS-CoV-2 infection can initiate the ATR DNA damage response. This may cause both the ATR and Chk1 transcripts to be overexpressed, as well as more phosphorylation of the Chk1 protein [15]. Our data aligned somewhat with a recent study by Gioia et al. (2023), which suggested that SARS-CoV-2 infection can lead to Chk1 degradation, thereby resulting in a shortage of Chk1-derived dNTP. We believe that anti-SARS-CoV-2 vaccination prior to or post COVID-19 may tilt the balance of DDR pathway downstream effectors and their phosphorylation. For instance, SARS-CoV-2 infection alone caused a decrease in the level of Chk1 kinase [16]. According to Ntouros *et al*. (2021), the anti-SARS-CoV-2 vaccine raises type I IFN levels, oxidative stress, and reversible DNA damage [17]. If the virus and the vaccine alone can generate such off-target effects, the simultaneous occurrence of both incidences in the same individuals may generate combined stress that causes deeper impacts at subcellular levels. Our results showed that TP53BP1 levels were slightly lower in people who were infected with SARS-CoV-2 compared to the control group. They were also significantly higher in people who had been exposed to SARS-CoV-2 before getting the vaccine compared to people who were infected or vaccinated (Figure 2). New research using cell models showed that SARS-CoV-2 can stop DNA damage repair by changing the way cell cycle regulators and tumor suppressor proteins like BRCA1 and TP53BP1 are recruited [16,18,19]. As in Chk1, previous infections and vaccinations in the same individuals may trigger more stress than each one can cause independently. Scientists have found that a big part of SARS-CoV protein 3 (nsP3) is the same as a big part of human ADP-ribosylhydrolases MacroD1 and MacroD2 [20]. These domains are involved in viral replication within the host cells [21]. For DNA damage repair to work, ADP-ribosylhydrolases need to be present to undo the ADP-ribosylation that happens after translation [22]. Therefore, it is possible to utilize these macrodomains in both host cells and the virus as therapeutic targets. Small inhibitors have been created to target macrodomains in cancer cells with inherited mutations [23,24]. This stops DNA damage repair and tumor growth by causing apoptosis. Furthermore, as these domains are necessary for viral replication, targeting them to inhibit virus invasion may be a unique approach to treating COVID-19

patients [21,25]. On the one hand, previous studies along with our data have revealed the potential roles that p53 and Chk1 play in SARS-CoV-2 and host-cell interactions. On the other hand, others have identified relevant targets that could potentially mitigate the risks associated with viral progression. Deltex E3 Ubiquitin Ligase 3L (DTX3L) changes the amount of p53 by ubiquitinating the C-terminal domain of p53 [26]. This happens after P53 and poly ADP-ribose polymerase 1 are found together in DNA damage sites. Another potential target is polo-like kinase 1 (PLK1), a protein kinase that plays a crucial role in regulating the cell cycle. Poly(ADP-)ribose forms a direct binding with PLK1, degrading its enzymatic activity. Meanwhile, Chk1 phosphorylates PLK1 and inhibits its interaction with the poly (ADP) ribose [27]. Therefore, by targeting DTX3L or PLK1 in the right manner, it is possible to modify signaling pathways that may culminate in the inhibition of viral replication. The long-term effects of COVID-19 are linked to the activity of P53 and Chk1. A lot of research shows that both P53 and Chk1 help keep the genome stable, and changes in their levels are linked to the development of several types of cancer [28,29]. Despite the previously mentioned information about SARS-CoV-2 and its interactions with host cells, there is currently no evidence to support the risk of the virus or its vaccines diminishing the functions of DDR proteins or jeopardizing DNA integrity, which could lead to genomic instability and/or the onset of diseases like cancer. However, further investigation is necessary to address these inquiries. Regarding the anti-SARS-CoV-2 IgG levels in the study participants, our data shows that there is no positive correlation between the groups. However, individuals who were infected prior to vaccination showed a slight increase in anti-SARS-CoV-2 IgG levels, especially when compared to those who had not received vaccination. For critically ill patients with infectious diseases, receiving passive immunization is considered an essential and time-saving treatment method. Unfortunately, there haven't been any reports of specific neutralizing monoclonal antibodies for SARS-CoV-2. It is possible that the presence of IgG antibodies against SARS-CoV-2 in the participants' sera is a result of previous exposure to common cold viruses from known coronavirus strains. The fact that SARS-CoV-2 and SARS-CoV are closely related viruses with a notable degree of sequence similarity in their spike (S) proteins [30] supports this idea. Antibodies from people who have recovered from SARS or from animals that have been infected with SARS-CoV-2 have been shown to stop the infection by blocking the S protein's ability to bind to and infect cells [31]. A worrying new study suggests that cross-reactive antibodies made by COVID-19 patients may help with functional diversity, which in turn helps the development of SARS-CoV-2 variants that the immune system can't recognize [32]. However, de Campos-Mata *et al*. (2024) and Yang and Du (2024) reported promising findings about small structural antibodies (or nanobodies) and their potential role in enhancing the ability to neutralize SARS-CoV-2 variants [33,34]. More studies, especially *in vivo* ones that mimic the natural microenvironment of a living host, are required to confirm our results and dive deeper into the COVID-19-related health problem details. Furthermore, it is crucial to address the phosphorylation levels in DDR proteins both before and after SARS-CoV-2 infection to evaluate possible chemical agents that may interfere with signaling pathways involved in these processes.

Conclusions

Both SARS-CoV-2 infection and the anti-SARS-CoV-2 vaccine may have an adverse effect on DDR-associated proteins in vitro. As the SARS-CoV-2 infection decreases, the combination of past infection and immunization increases the expression of Chk1 and TP53BP1. We hypothesize that the virally infected host cell tries to limit the viral infection and defend its DNA, which activates the DDR response. Furthermore, our data indicates that post-infection immunization boosts the generation of anti-SARS-CoV-2 IgG antibodies in COVID-19 survivors. Although certain studies have suggested that off-target adverse effects may be reversible, more study is needed to address these issues.

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Conflict of interests

No conflict of interest was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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