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RESEARCH ARTICLE

Association of *p53* codon 72 polymorphisms with expression status of hormone receptors like ER, PR, and HER-2 in invasive ductal breast carcinoma in Bangladeshi women

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Abstract

Background: There is a lack of data on prevalent genetic factors among female breast cancer patients from Bangladesh. *p53*, a suppressor gene, is crucial in breast cancer's aetiology. This study aimed to investigate the association between *p53* codon 72 polymorphisms and invasive ductal breast carcinoma (IDC) in Bangladeshi women.

Methods: This study included 203 formalin-fixed paraffin-embedded specimens of histologically confirmed IDC, with immunohistochemical analyses for ER, PR, and HER-2 status from November 2021 to October 2022. The specimens were collected from one laboratory in each of the Dhaka and Chattogram cities. *p53* codon 72 genotypes were detected using PCR-RFLP.

Results: Most patients (77%) were aged 41–60 years. All cases were IDC, with grade II (79.3%) and stage II being most prevalent (82%). ER-positive tumours were observed in 65.5% of patients, while 69% tumours were PR-negative and HER-2-negative. The GC (Arg/Pro) genotype was predominant (58.6%), followed by CC and GG (20.7% each). Statistically significant associations were found between the GC genotype and size less than 5 cm ($P<0.01$), axillary lymph node metastasis ($P<0.01$), and PR-negative tumours ($P=0.02$). Patients with GC+GG genotypes had higher odds of axillary lymph node metastasis (age, tumour grade and tumour stage adjusted odds ratio 21.8; 95% confidence interval 7.0–67.9), PR-negative tumours (aOR 0.2; 95% CI 0.1–0.6) and HER-2 negative tumours (aOR 0.3; 95% CI 0.1–0.9).

Conclusion: Our study suggests that the Arginine allele at *p53* codon 72, in either homozygous or heterozygous form, is associated with more aggressive IDC features in Bangladeshi women, including axillary lymph node metastasis and hormone receptor negative tumours.

Key messages

The study suggests that the presence of the Arginine allele in heterozygous (GC) or homozygous (GG) forms at codon 72 of the *p53* gene is associated with an increased risk of axillary lymph node metastasis, as well as PR-negative and HER-2-negative tumours, in female patients with invasive ductal breast carcinoma in Bangladesh. No significant link was found between this polymorphism and the ER status of the tumour.

Introduction

The Breast cancer, characterised by the malignant proliferation of epithelial cells within the ducts or lobules of the breast, is the most common malignancy in women, accounting for roughly one-third of all cancers in women worldwide [1]. Several risk factors have been identified, including some reproductive factors (age at menarche, menopause, first pregnancy, breastfeeding, and parity) and non-reproductive factors (menopausal hormone therapy, family history of cancer, body mass index, alcohol intake, and others) that are linked with breast cancer risk [2]. Besides these, women are adopting new lifestyles and undergoing significant demographic transitions. The higher incidence rates of breast cancer may also be due to genetic risk factors, which are still less studied.

Breast cancer is linked to various types of somatic genetic alterations, including mutations in oncogenes and suppressor genes [3]. The TP53 (*p53* gene, chromosome 17p13) protein function is altered in all cancers, including breast carcinomas, in approximately 20–40% of cases, depending on size and stage of disease [4]. The human tumour suppressor gene *p53* encodes a transcription factor that plays a central role in maintaining cellular integrity by inhibiting cell growth and stimulating apoptosis in response to DNA damage [5]. A common single-nucleotide polymorphism (SNP) occurs at the second position of codon 72 in exon 4 (rs1042522: CCC to CGC, Arg72Pro polymorphism), leading to an amino acid substitution of Proline (Pro) with Arginine (Arg) in the Pro-rich region of TP53 protein [6]. The distribution of the Pro and Arg alleles across three genotypic forms—CC (Pro/Pro), GG (Arg/Arg), and GC (Arg/Pro)—largely depends on the ethnic composition of the population studied [7]. The association of *p53* polymorphism at codon 72 and breast cancer development has been studied, but results have been controversial and not conclusive. Several studies have reported a significant association between the *p53* codon 72 polymorphism and breast cancer risk [7, 8, 9]. whereas others have identified no such association [10, 11]. In some studies, the Pro-allele has been associated with increased breast cancer risk [12, 13]. Other studies found the homozygous Arg-allele (GG genotype) associated with breast cancer predisposition [14, 15, 16]. Yet other studies, including most of the newer and larger studies and meta-analyses, did not detect any association of the Arg72Pro polymorphism with breast cancer risk [17, 18]. These discrepancies are attributed to the failure to determine the mutational status of *p53* in the study populations and/or the observed latitudinal differences in allele frequency [19].

The study of genetic influences in breast cancer is complex. Careful case selection is important, taking into account ethnic homogeneity, disease phenotype, and exposure to environmental risk factors. Targeted breast cancer management strategies may require not only molecular profiling but also knowledge of an individual's genetic susceptibility to develop metastatic disease. There is still a great deal more that needs to be discovered and understood before this type of genetic knowledge will find a valid place in the clinical care of individuals and families with breast cancer. However, such genetic study data regarding the association of *p53* codon 72 gene polymorphism with (a) invasive ductal breast carcinoma (IDC), and (b) hormone receptor expression status, i.e. estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2) of the IDC patients, are inadequate in Bangladesh. As *p53* mutations are potential prognostic and predictive markers, as well as targets for therapy [20, 21]. this study aimed to investigate the association of *p53* codon 72 polymorphisms with IDC in adult females and to assess the effect of this polymorphism on the ER, PR and HER-2 expression status of the tumour.

Methods

Breast tissue specimens

This study is based on formalin-fixed paraffin-embedded (FFPE) specimen blocks randomly selected from two institutions with similar competency in Bangladesh: the Department of Pathology at Bangladesh Medical University, Dhaka, and the Care Investigation Histopathology and Cytopathology Laboratory in Chattogram, from November 2021 to October 2022. A simple random sampling of specimens was conducted using the sampling frame maintained by the laboratories' registry.

We short-listed 374 registered IDC cases from the histopathology registries. The inclusion criteria were: (i) Bangladeshi adult females diagnosed with unilateral IDC for the first time; (ii) availability of data on expression status for three hormone receptors important for IDC diagnosis and treatment, i.e. ER, PR and HER-2; (iii) availability of other clinical and pathological data, *e.g.* patients' age, tumor size, axillary lymph node metastasis, number of lymph node involved; (iv) the patients did not receive any extensive treatment for IDC prior to surgical removal of the tumour. The exclusion criteria were: (i) IDC cases other than adult females; (ii) recurrence or patients had other cancers as primary disease; (iii) core FFPE block is not available for research; (iv) FFPE blocks not in good condition for taking microtome sections for DNA isolation, histopathology imaging for the study, and immunohistochemical analysis, (v) necesssary clinical and pathological data of the cases are not available. Finally, a total of 203 cases were selected for the study.

FFPE tissue blocks, along with relevant clinical and pathological data of the 203 cases, were collected from the mentioned institutions for the analyses of this study. Here, the presence of hormone receptor expression of ER, PR and HER-2 was recorded as positive, and the absence of such expression was recorded as negative for corresponding hormone receptors. Polymorphism detection of *p53* codon 72 and data analyses were done at the Functional Genomics and Proteomics Laboratory of the Department of Genetic Engineering and Biotechnology, University of Chittagong.

Isolation of genomic DNA

Multiple sections (4-8 sections) each measuring 2-3 µm in thickness were taken from the selected FFPE

blocks using a semi-automatic microtome. These sections were used as starting material for DNA isolation. Isolation of genomic DNA from the sections was done according to the manufacturer's protocol of GeneJET FFPE DNA Purification Kit (Thermo Scientific, USA) [22]. After isolation, the DNA were preserved at -20°C for further analysis.

Polymerise chain reaction (PCR) for *p53* codon 72 region

Nested enrichment PCR and subsequent restriction enzyme digestion were done for the detection of *p53* c72 SNPs. The PCR primers used in this experiment were previously described [23, 24]. For the first round PCR, the primers (IDT, Singapore) F1: 5′-GCTCTTTTCACCCATCTACAG - 3′ and R1: 5′-TGAAGTCTCATGGAAGCCAGC - 3′ were used along with 100ng DNA from FFPE block. For the second round PCR, F2: 5′ - TCCCCCTTGCCGTCCCAA - 3′ and R2: 5′ - CGTGCAAGTCACAGACTT - 3′ primers (IDT, Singapore) were used along with 2µL of 10X diluted PCR product of first round PCR. In both first and second round PCR reactions, the reaction mixture contained 1X GoTaq® Flexi reaction buffer (Promega, USA), 2mM MgCl2 (Promega, USA) 0.1mM of each dNTPs (Sigma, USA), 1U GoTaq® Flexi DNA Polymerase (Promega, USA), and Nuclease free water (Invitrogen, USA) was used to make the reaction volume upto 25µL. The PCR thermal cycler (Qantarus, UK) profile included Initial denaturation at 95°C for 5 minutes, 35 (first round) and 30 (second round) cycles of denaturation at 95°C for 30 seconds, annealing at 58.5°C for 30 seconds, and elongation at 72°C for 1 minute.

Restriction fragment length polymorphism (RFLP) for *p53* codon 72 polymorphisms

RFLP for *p53* codon 72 polymorphism detection was done by digesting 10µL of second round PCR products by 2U of Bsh1236I restriction enzyme which contains the restriction site 5′-CGCG-3′ (BstUI, Thermo Scientific, USA) in 0.67X Buffer R (Thermo Scientific, USA) and nuclease free water (Invitrogen, USA) was used to make reaction volume upto 25µL. The temperature profile for restriction digestion consisted of incubation at 37°C for 4 hours and 30 minutes, followed by inactivation at 65°C for 20 minutes. All the restriction digestion reactions were carried out in a heating block (WiseCube, Daihan Scientific, Korea).

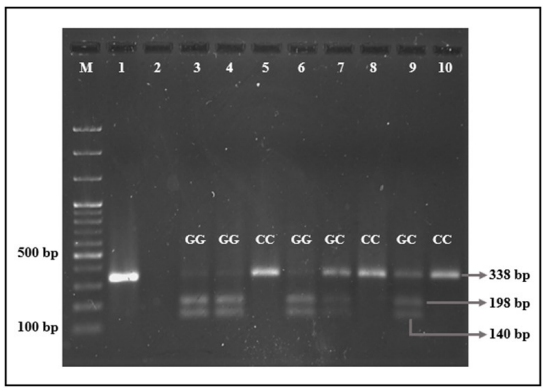

Figure 1 *p53* codon 72 polymorphism detection in invasive ductal breast carcinoma patients. Single-nucleotide polymorphism variants are marked in corresponding lanes. Band sizes are mentioned in base pairs (bp). Lane M: DNA size marker (100bp+ DNA marker). Lane 1: undigested *p53* codon 72 PCR product (338 bp). Lane 2: Blank. Lanes 3-10: BstUI digested *p53* codon 72 PCR products. Lanes 5, 8 and 10: presence of CC homozygous SNP variant caused the absence of BstUI recognition site and thus remained uncut (338 bp) Lane 3-4 and 6: presence of homozygous GG SNP variant caused complete digestion of PCR product into 198 bp and 140 bp fragments. Lanes 7 and 9: presence of heterozygous GC SNP variant caused incomplete digestion of PCR products and produced 338 bp, 198 bp and 140 bp bands.

Agarose gel electrophoresis and detection of *p53* codon 72 polymorphisms

2% w/v low electroendosmosis (Low EEO) agarose (Promega, USA) was completely dissolved in 1X TAE buffer (pH 7.9) by using a high-temperature quick-dissolve technique with a microwave oven. Five microliters of Safe Dye (AdBio Solutions, South Korea) were added while preparing the gel for visualisation of DNA bands in a UV Gel Documentation System (Vilber, France). In each agarose gel preparation, a thickness of 3-4 mm was maintained for clearer visualisation. Horizontal submarine electrophoresis was done in either Biometra Compact XS Electrophoresis Apparatus (Analytic Jena, Germany) or Hoefer HE 33 Mini Electrophoresis Unit (Thermo Fisher Scientific, USA) with the corresponding power supply kits. 1X TAE (pH 7.9) was used as running buffer during electrophoresis. 10 µL of PCR or PCR-RFLP products were loaded in the wells along with 2 µL 100 bp DNA size marker (Promega, USA) in the left-most well. Electrophoresis was carried out for the required durations with constant voltage (55V) control (Figure 1).

Statistical analyses

A Chi-squared test was performed to determine whether there was any significant deviation from the expected distribution of *p53* codon 72 genotypes among the IDC samples, considering variables such as tumour size, lymph node metastasis, and hormonal receptor expression status (ER, PR, and HER-2). To find the most influential *p53* codon 72 polymorphic variant upon pathological status and ER, PR, HER-2 expression status, by applying univariate and multivariate logistic regression, which are usually done in single-nucleotide polymorphism (SNP) association studies to calculate odds ratios and their 95% confidence intervals. In the univariate analysis, the SNP counts of CC homozygotes versus GC+GG genotypes (*i.e.* reflecting proline versus arginine alleles) were compared according to various parameters. Multivariate logistic regression analysis was conducted to adjust the results according to patients' age, tumour grade and tumour stages. $P<0.05$ was considered statistically significant. All data were analysed with SPSS Software v25.

Results

Pathological status of the cases

The age of the 203 patients spanned from 21 to 94 years. However, there were 99 patients from the 41–60 years age group (48.87%) (Table 1). All the samples were confirmed as invasive ductal carcinoma. Histopathological observation revealed that most patients (79.3%) had tumour grade II, and 82% were in

Table 1 Clinical and pathological details of the study patients (n=203)

Variables	Number (%)
Age (Years)	
21–40	63 (31.0)
41–60	99 (48.8)
61–94	41 (20.2)
Tumor grade	
Grade I	21 (10.3)
Grade II	161 (79.3)
Grade III	21 (10.3)
Tumor staging	
T1 (<2 cm)	8 (3.9)
T2 (2–5 cm)	167 (82.3)
T3 (>5 cm)	28 (13.8)
Axillary lymph node status	
Sinus histiocytosis	67 (33.0)
Metastatic ductal carcinoma	98 (48.3)
Follicular hyperplasia	38 (18.7)
Number of lymph node involved (n=98)	
N1 stage (1–3 lymph nodes involved)	63 (64.3)
N2 stage (4–9 lymph nodes involved)	28 (28.6)
N3 stage (>10 lymph node involved)	7 (7.1)
Estrogen expression	
Positive	133 (65.5)
Negative	70 (34.5)
Progesterone expression	
Positive	63 (31.0)
Negative	140 (69.0)
HER2 expression	
Positive	63 (31.0)
Negative	140 (69.0)
p53 codon 72 SNP	
CC genotype	42 (20.7)
GC genotype	119 (58.6)
GG genotype	42 (20.7)

tumour stage II. Forty-eight per cent had metastatic ductal carcinoma in their axillary lymph nodes, and 64.3% were in N1 stage (1-3 lymph nodes involved).

ER, PR and HER-2 expression status

Immunohistochemical analysis revealed that most of the cases (65.5%) were positive for ER expression. Whereas most patients were negative for PR and HER-2 expression (Immunohistochemical features are shown in Figure 2).

p53 codon 72 genotype frequencies and their associations

The GC heterozygote variant was the most common among the study patients, accounting for 58.6%. Both CC and GG homozygous variants were found in equal numbers; that is, 20.7% of patients had the CC homozygote variant, and 20.7% had the GG homozygote variant. It was suggested that the GC genotype might be associated with the disease conditions in this study population.

A significant association was observed between the *p53* codon 72 polymorphic genotype and clinical parameters, including tumour size, axillary lymph node metastasis, and PR expression (Table 2). The Arg/Pro heterozygous allele (GC genotype) was significantly associated with tumours less than 5 cm in size ($P<0.01$), axillary lymph node metastasis ($P<0.005$). Furthermore, a statistically significant association was noted between the GC genotype and PR-negative tumours ($P=0.02$). No significant associations were observed between the *p53* codon 72 genotypes and ER or HER-2 status.

From logistic regression analyses (Table 3), it was found that patients carrying Arg/Pro+Arg/Arg alleles (GC+GG genotypes) were significantly more likely to be presented with axillary lymph node metastasis compared to homozygous Pro allele (CC genotype) carriers. The crude OR was 6.5 (95% CI 2.7–15.5), while the aOR increased markedly to 21.8 (95% CI 7.0 –67.9), indicating a strong probability. No significant association was found between the *p53* codon 72 polymorphism and the ER expression status. The OR was 0.9 (95% CI 0.5–1.9), and the aOR was 0.6 (95% CI 0.3–1.4). In contrast, PR-negative tumours were common among GC+GG genotype carriers. The OR was 0.4 (95% CI 0.2–0.7), and the aOR was 0.2 (95% CI 0.1–0.6), suggesting a strong probability of finding these genotypes in ER-negative tumours. There was no significant representation of any *p53* codon 72 genotype according to HER-2 expression status in univariate logistic regression (OR 0.9; 95% CI 0.4–1.2), whereas, adjusted multivariate logistic regression analysis revealed that the Arg/Pro+Arg/Arg alleles (GC+GG genotypes) were significantly more presented in HER-2-negative tumors (aOR 0.3; 95% CI 0.1–0.9).

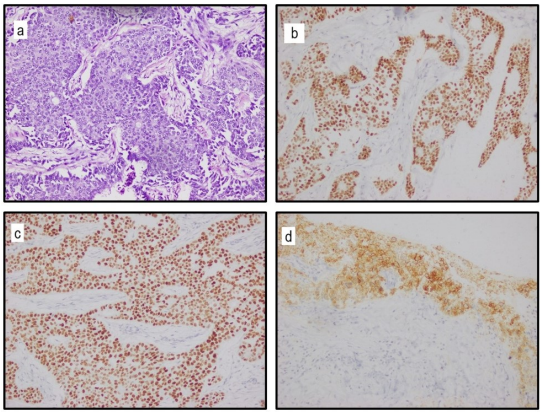

Figure 2 Immunohistochemical evaluation for ER, PR and HER2 expression after histological confirmation of invasive ductal breast carcinoma grade II. The patient was a 45-year-old female (case no.: 26). (a) Histological image of breast tissue sections of a showing features of invasive ductal carcinoma; (b), (c) and (d) are immunohistochemical images showing positive staining for ER, PR and HER2 expressions, respectively.

Discussion

This study investigated the association between *p53* codon 72 polymorphism and IDC in Bangladeshi adult women, with a focus on clinicopathological features and hormone receptor expression status. The *p53* codon 72 SNP results in either a proline or arginine residue in the TP53 protein, influencing its tumour suppressor functions and apoptotic capacity [25, 26]. Understanding this polymorphism's role could improve insights into breast cancer susceptibility, progression, and prognosis.

Table 2 Distribution of *p53* codon 72 CC homozygotes (Pro/Pro) versus GC heterozygotes (Arg/Pro) across variables

Variables	Pro/Pro (CC) (n=42)	Arg/Pro (GC) (n=119)	*P*
Tumor size (cm)			
<5	21	98	<0.001
≥5	21	21	
Axillary lymph node metastasis			
No metastasis	35	49	0.01
Metastatic ductal carcinoma	7	70	
Expression of estrogen receptor			
Negative	14	49	0.46
Positive	28	70	
Expression of progesterone receptor			
Negative	21	84	0.02
Positive	21	35	
Expression of human epidermal growth factor receptor 2			
Negative	28	91	0.23
Positive	14	28	

Our findings showed that Bangladeshi women aged 41–60 years exhibited the highest IDC prevalence. This observation aligns with data from the Polish population, which demonstrated a linear increase in breast cancer incidence among women aged 40–59, followed by a plateau in those aged 70 and above [27]. Similarly, studies conducted by Acharya *et al.* and Pathak *et al.* reported the 41–60-year age group as the most commonly affected by breast cancer [28, 29]. This trend may be associated with hormonal fluctuations during the peri- and postmenopausal periods, which are known to influence breast cancer risk [27].

Numerous studies have assessed the role of the Arg72Pro polymorphism in cancer risk, but findings have been inconsistent across populations [26]. In our study, the heterozygous Arg/Pro (GC genotype) was the most common, observed in 58.6% of patients. This contrasts with a recent study from Brazil involving 96 individuals, which reported a high prevalence of the Arginine allele (68%), whereas we found equal frequencies of the Arginine and Proline alleles [30]. Similar results were observed concerning the involvement of the heterozygous Arg/Pro (GC genotype) variant and an increased risk of breast cancer in the North Indian population [31]. Another study reported that Proline homozygosity (CC genotype) at *p53* codon 72 is associated with decreased breast cancer risk in Arabian women [32].

The current study's findings suggest that the presence of Arg allele (GC+GG genotypes) is significantly associated with an increased risk of axillary lymph node metastasis. Some studies have shown that the mechanism of breast carcinogenesis and its progression is associated with alterations in the expressions of ER, PR, and HER-2 [33]. Notably, a significant association was observed between the Arg-containing genotypes and PR-negative tumours, suggesting a possible link between the Arg allele and hormone-independent tumour biology. This result is consistent with findings from studies in Asian populations, where the Arg allele was more frequently associated with hormone receptor–negative tumours, which tend to be more aggressive and less responsive to endocrine therapy [33, 34].

In the case of HER-2 expression, the Arg/Pro+Arg/Arg alleles (GC+GG genotypes) were initially not associated in the univariate logistic regression analysis. However, after adjusting for patients' age, tumour grade and tumour stages, a significant association was found between GC+GG genotypes and HER-2-negative tumours. This observation is novel in our study as previous studies found no significant association [35, 36], and requires further investigation. No significant association was found between *p53* codon 72 polymorphism and ER status, which contrasts with some previous reports suggesting a genotype-specific interaction with ER expression [37]. This discrepancy may be due to population-specific genetic backgrounds, sample size, or environmental co-exposures such as heavy metals, which are particularly relevant in the Bangladeshi context. Thus, discrepancies between our findings and those of other studies may reflect population-specific genetic and environmental interactions.

Our findings suggest that patients who present a heterozygous genotype and/or Arginine allele at codon 72 of the *p53* gene may have a susceptibility towards breast cancer along with axillary lymph node metastasis. This could serve as a potential biomarker

Table 3 Likelihood of *p53* codon 72 polymorphic variants and axillary lymph node metastasis and hormone receptor expression status (ER, PR and HER-2) of patients

Variables	Genotypes		Odds ratio (95% confidence interval)	Adjusted[a] odds ratio (95% confidence interval)
	Pro/Pro (CC)	Arg/Pro +Arg/Arg (GC+GG)		
Axillary lymph node metastasis				
No	35	70	6.5 (2.7–15.5)[b]	21.8 (7.0–67.9)[b]
Yes	7	91		
Estrogen Receptor expression status				
Negative	14	56	0.9 (0.5–1.9)	0.6 (0.3–1.4)
Positive	28	105		
Progesterone Receptor expression status				
Negative	21	119	0.4 (0.2–0.7)[b]	0.2 (0.1–0.6)[b]
Positive	21	42		
HER2 expressions status				
Negative	28	112	0.9 (0.4–1.2)	0.3 (0.1–0.9)[b]
Positive	14	49		

[a]Multivariate logistic regression was done for adjustment of age, tumour grade and tumour staging; [b]Statistically significant at 5% or smaller label

for prognosis. The significant association of this genotype with PR-negative and HER-2-negative tumours highlight the potential clinical relevance of this SNP in predicting breast cancer diagnosis and guiding personalised treatment strategies and outcomes. Further investigations in larger and more diverse cohorts are needed to validate our findings.

Limitations

Some FFPE archival samples were excluded due to poor performance in the DNA isolation procedure or the unavailability of all required data, including clinical data and histology reports. Due to the limited funds, the researchers had to restrict the number of cases analysed; as a result, more cases from around Bangladesh could not be included. Ultimately, the DNA sequencing of the 203 specimens in this research work could be more informative.

Conclusion

This study found that *p53* codon 72 polymorphism is significantly associated with histological and immunohistochemical features of IDC. However, for further association studies with this polymorphism and confirmation of clinical implication, the following recommendations can be considered: Case-control studies involving newly diagnosed, unilateral and bilateral breast cancer patients with age-matched healthy controls could provide more reliable information. Prognostic studies of various treatment regimens could further substantiate the evidence.

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Author contributions

Conception or design of the work; or the acquisition, analysis, or interpretation of data for the work: MZR, MJA. *Drafting the work or reviewing it critically for important intellectual content:* MZR, MJA, AB, MARR, FBW, IIS, SRAS, LK, MAF. *Final approval of the version to be published:* MZR, MJA, AB, MARR, FBW, IIS, SRAS, LK, MAF. *Accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved:* MZR, MJA, MAF.

Conflict of interest

We do not have any conflict of interest.

Data availability statement

We confirm that the data supporting the findings of the study will be shared upon reasonable request.

Supplementary file

None

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