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Original Research

Blood, saliva and urine maresin-1 and malondialdehyde may be useful biomarker in patients with polycystic ovary syndrome: a prospective study

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Abstract

Background: Maresin-1 (MaR1) plays a major role in many inflammatory disorders. Polycystic ovary syndrome (PCOS) aside from a hormonal disorder, an inflammation might also contribute to PCOS and its metabolic associations. Therefore, the purpose of this prospective study first time was to find out the blood, saliva and urine levels of MaR1 in PCOS patients and evaluate the correlations with other metabolic and hormonal parameters. **Methods**: Thirty PCOS patients and 30 matched healthy controls were enrolled to prospective case control study. Blood, urine and saliva samples were simultaneously collected from participants after overnight fasting. MaR1 levels in blood, urine and saliva samples were determined by enzyme-linked immunosorbent assay. Ferriman-Gallwey score, anthropometric, hormonal and some other metabolic parameters were also recorded. Regression analysis was preformed to find out the relationship between MaR1, C-reactive protein (CRP) and malondialdehyde (MDA), and hormonal and metabolic parameters. **Results**: Patients with PCOS compared with control women had higher MDA and CRP and decreased MaR1 levels. Blood, urine and saliva MDA and MaR1 levels were similar and indicated parallel decrease or increase in the PCOS and control groups. Furthermore, regression analysis indicated that blood CRP and MDA was positively associated with luteinizing hormone (LH) and fasting insulin (FI) in PCOS group ($p < 0.05$) while blood, urine and saliva MaR1 was negatively associated with CRP and MDA. **Conclusions**: Present results (MaR1, CRP and MDA together) in case of PCOS suggests that decreased MaR1 and elevated MDA and CRP levels in patients with PCOS and may be considered as a useful early biomarker (especially MaR1) in diagnosis of PCOS disease that has not been previously reported and regular monitoring of their levels could be helpful in clinical decisions.

Keywords: urine; saliva; maresin-1; malondialdehyde; polycystic ovary syndrome

1. Introduction

Polycystic ovary syndrome (PCOS) is an endocrinal and metabolic disorders with a prevalence of 6–14% of women reproductive age [1,2], and is linked with metabolic disorders, including insulin sensitivity that is decreased by 35%–40% in females with PCOS, independent of obesity, a decrease similar in magnitude to that seen in diabetes [3]. Also numerous studies reported that number of abnormal hormone levels (androgen, anti Müllerian hormone (AMH), cortisol, insulin, and prolactin) neurotransmitters (dopamine), peptides, proteins, and glucose are also contributed to PCOS manifestation and its metabolic associations [4–7]. Furthermore, PCOS is associated CRP compared to those without the condition, suggesting CRP a marker of low-grade inflammation in PCOS that is independently related to insulin resistance [8].

Increase in both low-grade chronic inflammation and insulin resistance in women with PCOS is associated with increased central fat excess [9]. Normoweight patients with PCOS have higher lipid accumulation in visceral deposit and lower subcutaneous lipid in gluteofemoral area [10]. Lipid peroxidation is forming in the polyunsaturated fatty acid side chains of the plasma membrane or that of any organelle that bears lipids [11]. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells, mostly existing in the enol form in the biological fluids [12]. MDA elevates significantly in the PCOS patients compared with the normal [13].

Maresin1 (MaR1or 7*R*, 14*S*-dihydroxy-4*Z*,8*E*,10*E*,12*Z*,16*Z*,19*Z*-docosahexaenoic acid with chemical formula $C_{22}H_{32}O_4$) is a novel lipid mediator biosynthesized from docosahexaenoic acid (DHA) by mainly macrophages (play a major role in resolving inflammation) that regulates acute inflammation against several animal models, including lung fibrosis, sepsis, obesity, brain ischemia, in abating neuropathic pain, and

nonalcoholic steatohepatitis [14–19]. This molecule also has a role in the process of tissue regeneration and pain control [20]. It has been reported that MaR1 is associated with many physiopathological conditions such as the development of many prevalent chronic diseases, including insulin resistance, and type 2 diabetes [21]. MaR1 was detectable in the plasma and urine [14,21,22]. It was also detected in the synovial fluid taken from the joins of patients with rheumatoid arthritis [23].

As stated above the pathogenesis of PCOS is very complicated and its underlying basis mechanism is still completely unknown yet. Also accumulating evidence suggests that MaR1 exhibits protective and anti-inflammatory effects in some diseases, including colitis pneumonia, and promotes wound healing in planaria worm model, healing of vascular injury and socket bone regeneration [24–27];however, its role in PCOS has not investigated yet. It has been previously reported that there is also an association between PCOS and inflammation biomarkers (C-CRP) and MDA). Even though MaR1 is novel lipid biomarkers, there is no study available that examined whether MaR1 and MDA relationship present in the patient with PCOS. Therefore, this current prospective study was to examine whether blood, urine and saliva MaR1or MDA are associated in patients with PCOS when compared with its counterpart control subjects. Also this study wants to find out whether CRP or MaR1 which one is better inflammation biological markers in case of patients with PCOS.

2. Materials and methods

Overnight fasting biological samples (5 mL blood, 2 mL urine and 2 mL saliva) were simultaneously collected from 30 body mass index (BMI) and age matched PCOS subjects and 30 control subjects at Nigde Omer Halisdemir University (NOHU) Training and Research Hospital Department of Obstetrics and Gynecology. The study protocol was accepted by the NOHU Non-Interventional Clinical Research Ethics Committee (Date: 2021, issue no: 12) and informed consent was taken from each participant subjects. Participants were also asked not to drink, except water, or chew gum for the same period. Biological samples were centrifuged at 4000 rpm for 5 minutes. Then, the liquid components (blood, urine, saliva) transferred into a clean Eppendorf tube using a Pasteur pipette. Then, they are stored at –80 ℃ until study. The BMI was calculated by dividing a person's weight in kilograms by the square of height in meters.

2.1 Inclusion and exclusion criteria

PCOS has been diagnosed according to Rotterdam consensus criteria as details given below: Two of the following criteria, in addition to exclusion of related disorders (pregnancy, thyroid dysfunction, primary ovarian insufficiency; hyperprolactinemia, and nonclassical congenital adrenal hyperplasia and hypothalamic amenorrhea): polycystic ovaries (12 or more follicles in each ovary, each follicle measuring 2–9 mm in diameter and/or ovarian volume 10 mL, one polycystic ovary is sufficient for the diagnosis), oligo-or anovulation and/or polycystic ovarian morphology; clinically diagnosed as oligo-/amenorrhea (menstrual cycles less than 10 menstruations per year or longer than 35 days last) and clinical hirsutism (Ferriman-Gallwey score over 8) [28,29] and/or biochemical signs of hyperandrogenism (e.g., elevated levels of total or free testosterone) [30]. Also, any PCOS patients who had infectious diseases, obesity, vasculitis, rheumatologic diseases, ulcerative colitis, asthma, pneumonia, and chronic obstructive pulmonary disease were excluded from this study. Control group participants (they came to hospital for routine annual checking) who have no clinically any known diseases were invented for this study. From these peoples anyone who wanted voluntarily accepts our invitations for this study were used as a control groups.

2.2 Biochemical analysis

Blood CRP (mg/dL), LH (mIU/mL), estradiol (E2) (pg/mL), FSH (mIU/mL), FSH, dehydroepiandrosterone sulfate (DHEA-SO4) (μg/dL), total testosterone (nmol/L), fasting blood glucose (FBG) (mg/dL), insulin (lIU/mL) were obtained from participant hospital files. These above parameters were chosen from participant hospital files since they represent key metabolic features of PCOS. Insulin resistance (IR) was also calculated by using the Homeostatic Model Assessment-IR (HOMA-IR = fasting insulin $\times$ FBG/22.5) [31].

2.3 Assessment of biological fluid MaR1 concentrations

Human MaR1 (Cayman, Ann Arbor, MI, USA) ELISA is used to measure & quantify maresin-1 level in biological fluids in accordance with the study procedures specified in the catalogs. This kit was stored –80 ℃ until use. The coefficient of variation (CV) intra-assay precession value is 4.6 while % the coefficient of variation (CV) inter-assay precession value is 10.00. Minimum detection limit of MaR1's kit is 9.6 pg/mL. The automatic washer Bio-Tek ELX50 (BioTek Instruments, Winooski, Vermont, USA) was used for plate washing while the ChroMate, Microplate Reader P4300 instrument (Awareness Technology Instruments, Palm City, FL, USA) was used in absorbance readings at the wavelength of 405 nm and the content of MaR1 in the sample was calculated by standard curve. Human MaR1 was used to measure & quantify maresin-1 level in blood and saliva. However, whether this human MaR1 ELISA kit measures & quantifies MaR1 level accurately in blood and saliva as in urine, we have done precession assay according to published method previously [32].

2.4 Analysis of malondialdehyde

The principle of this method was based on the spectrophotometric measurement (at 532 nm) of the color that

Table 1. Results of assay validation of blood and saliva MaR1 (Cayman, Ann Arbor, MI, USA) ELISA kit.

MaR1	Precision (CV %)		Accuracy			DL (%)*
	CWR	CBR	Measured Value (pg/mL)	Certified Value (pg/mL)	Certified DL (%)	
Urine	7.5	2.1	507.79	400	100	98*
Urine	7.1*	5.2*	508.79*	400	100	93*
Blood	6.9*	7.3*	598.64*	NA	NA	104*
Saliva	7.2*	7.5*	543.73*	NA	NA	106*

CV, coeffient variation; DL, dilution linearity (1/4); NA, not available; CWR, certified within run; CBR, certified between run.

*, our lab results.

occurring during the reaction to thiobarbituric acid (TA) with MDA. Biological fluids MDA levels were analyzed for all study subjects by the double heating method. The concentration MDA was also assayed in the saliva of study subjects, as previously described (Khalili and Biloklytska 2008). Levels of TA reactive substances was calculated by the absorbance coefficient of MDA-TA complex and given in μmol/L. Assay sensitivity is 0.01 μmol/L. Intra-assay and inter assay CV are 12% and 15%, respectively.

2.5 Statistical analysis

Statistical analysis was done with Statistical Package for the Social Sciences (SPSS 22.0) software package (SPSS Inc., Chicago, IL, USA). The association between CRP, MDA and Mar1 and other potential covariates was analyzed by using Mann-Whitney (MW) or Kruskal-Wallis (KW) for comparisons of CRP, MDA and MaR1 levels between groups and Spearman's rho (ρ) for correlations between levels of CRP, MDA and Mar1 and continuous variables. Data were expressed as mean $\pm$ standard deviation. A probability (p) value of <0.05 was accepted a statistically significant difference.

3. Results

In this study, assay validation of MaR1 (Cayman, Ann Arbor, MI, USA) ELISA kit has been done. It has been seen that human urine MaR1 (Cayman, Ann Arbor, MI, USA) kit measure human saliva and blood MarR1 level as much as urine MaR1 level (Table 1). The clinical and endocrine characteristics of participants are shown in Table 2. Both the study populations were age and BMI-matched. As compared with the control, the mean LH, FSH, testosterone, androstenedione, fasting levels of insulin level were significantly elevated in the PCOS population (Table 2).

The comparisons of the blood, saliva and urine MaR1 levels between groups of PCOS patients and the control subjects are showed in Fig. 1. Fig. 1 indicated that blood, urine and saliva MaR1 concentrations were significantly were lower in patient with PCOS than that of control subjects blood, urine and saliva MaR1 concentrations. The comparisons of CRP levels between groups of patients and the healthy control subjects are also showed in Fig. 2. CRP levels were significantly higher when compared with control CRP values. The comparisons of the blood, urine and MDA levels between groups of patients and the control subjects are also showed in Fig. 3. Also Fig. 3 showed that blood, urine and MDA levels were significantly elevated in PCOS patient when compared with control subject's values. Furthermore there were no significant differences of blood, urine, saliva and MaR1 concentrations when compared with themselves (blood, urine and saliva) MaR1 concentration with and without PCOS subjects.

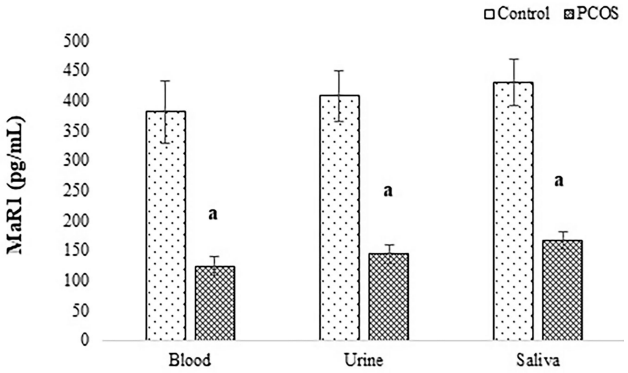

Fig. 1. Level of the inflammation product, MaR1, in blood, urine and saliva of patients with PCOS in comparison with control subjects. The differences between the controls and PCOS were significant at ($p < 0.001$).

Also, linear regression analysis revealed that a negative correlation were found between testosterone, LH, FSH, testosterone, androstenedione, HOMA-IR, and fasting levels of insulin and blood, urine and saliva MaR1 levels in the PCOS population (Table 2). Decreased blood, urine and saliva MaR1 are associated with increased levels androgens and excess hair growth (hirsutism) in women with polycystic ovary syndrome. Furthermore, there was a positive correlation were found between testosterone, LH, FSH, testosterone, androstenedione, FerrimanGallwey score, HOMA-IR, and fasting levels of insulin, and MDA and CRP levels in the PCOS population (Table 3). Blood urine and saliva MaR1 concentrations were also significantly corre-

Table 2. The statistics and comparison of demographic and some laboratory variables in control and polycystic ovary syndrome women.

Variables	Control (n: 30)	PCOS (30)	*p* values
Age (years)	28.4 ± 3.8	27.9 ± 3.1	0.9
BMI (kg/m^2)	22.52 ± 2.6	23.11 ± 2.9	0.8
Ferriman-Gallwey score	6.9 ± 1.1	9.34 ± 1.2	0.02
FBG (mg/dL)	94.08 ± 6.8	102.2 ± 6.9	0.82
DHEA-SO_4 (μg/dL)	128.4 ± 22.3	168.3 ± 32.2	0.07
Testosterone (ng/mL)	0.42 ± 0.16	0.74 ± 0.26	0.03
E2 (pg/mL)	81.2 ± 26.9	103.2 ± 26.6	0.01
FSH (mIU/mL)	6.67 ± 1.1	7.74 ± 1.6	0.09
LH (mIU/mL)	8.6 ± 1.44	16.3 ± 1.32	0.01
Insulin (µIU/mL)	11.33 ± 0.47	18.22 ± 0.74	0.03
HOMA-IR	2.92 ± 0.32	4.76 ± 0.49	0.01

BMI, body mass index; DHEA-SO_4, dehydroepiandrosterone sulfate; E2, estradiol; FBG, fasting blood glucose; FSH, follicle-stimulating hormone; HOMA-IR, Homeostasis model assessment of insulin resistance; LH, luteinizing hormone; PCOS, polycystic ovary syndrome.

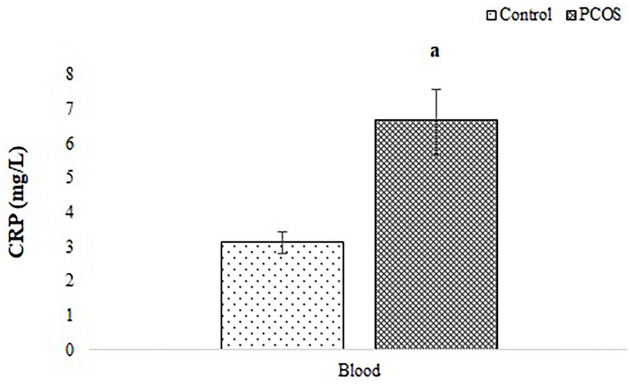

Fig. 2. Level of the inflammation product, CRP, in blood of patients with PCOS in comparison with control subjects. The differences between the controls and PCOS were significant at ($p < 0.05$).

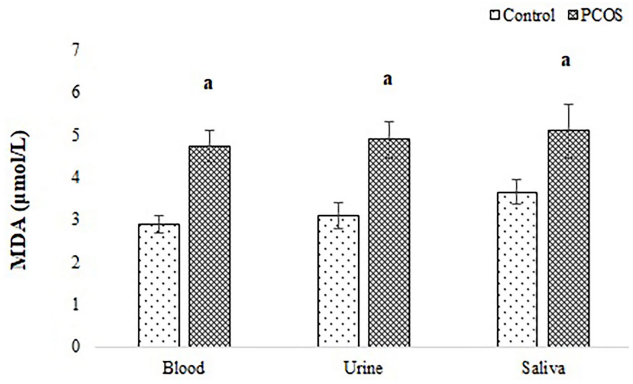

Fig. 3. Level of the lipid peroxidation product, MDA, in blood, urine and saliva of patients with PCOS in comparison with control subjects. The differences between the controls and PCOS were significant at ($p < 0.001$).

lated with parallel decrease ($r = 0.37$, $p = 0.03$, $r = 0.39$, $p = 0.03$ and $r = 0.35$, $p = 0.04$, respectively) in the PCOS population as well.

4. Discussion

In this prospective study of PCOS women, we found a significant association with the development of PCOS, including higher HOMA-IR and FBG, higher dehydroepiandrosterone sulfate, E2, FSH, LH, testosterone and Ferriman-Gallwey score. Our observations confirm the previous results reported by different authors, who demonstrated a strong link between metabolic parameters (e.g., FBG) and hormone levels and PCOS [7,33–35].

In addition, elevated levels of CRP compared with control women were found to be significant predictive factors for PCOS. Elevated levels of CRP in women with PCOS in this study might be a sign of chronic inflammation [36]. Previous some studies have also suggested that the association between CRP and PCOS may be a stronger sing for inflammation in PCOS patients [37–39] while previous studies found that there was a slight difference in CRP with and without PCOS [40,41]. These slight differences in some studies may be related to the factors such as BMI, presence asthma, pneumonia, and chronic obstructive pulmonary disease and assays used in them. Our study subjects were age and BMI matched subjects and also excluded from our study any participants who had asthma, pneumonia, and chronic obstructive pulmonary disease. Therefore, our elevated levels of CRP in women with PCOS could directly be associated with diseases of PCOS.

In this study it was also found that MDA levels in biological fluids of women with PCOS were significantly

Table 3. The correlation between MaR1, CRP, MDA and some hormonal level among polycystic ovary syndrome patients.

Parameters	MaR1		MDA		CRP	
	r value	*p* value	*r* value	*p* value	*r* value	*p* value
	–0.47*	0.01*	0.72*	0.00*	0.39*	0.05*
E2*	–0.49**	0.01**	0.57**	0.00**	NM**	NM**
	–0. 62***	0.00***	0. 44***	0.001***	NM***	NM***
	–0.37*	0.003*	0.38*	0.09*	0.46	0.05
FSH*	–0.39**	0.001**	0.31**	0.01**	NM**	NM**
	–0.29***	0.002***	0.46***	0.01***	NM***	NM***
	–0.57*	0.00*	0.40*	0.05*	0.43*	0.57*
LH*	–0.58**	0.00**	0. 44**	0.00**	NM**	NM**
	–0.72***	0.00***	0.53***	0.00***	NM***	NM***
	–0.54*	0.03*	0.33*	0.05*	0.56	0.00
Insulin*	–0.57**	0.02**	0.41**	0.00**	NM**	NM**
	–0.48***	0.03***	0.38***	0.01***	NM***	NM***
	–0.37*	0.01*	0.49*	0.01*	0.38*	0.05*
Testosterone*	–0.42**	0.01**	0.63**	0.00**	NM**	NM**
	–0.44***	0.01***	0.58***	0.00***	NM***	NM***

CRP, C-reactive protein; E2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; MaR1, maresin-1; MDA, malondialdehyde; NM, not measured.
*, Blood; **, Urine; ***, Saliva.

higher than that of control subjects. As known, MDA occurs naturally and is a marker for oxidative stress [42]. Here elevated levels of MDA in women with PCOS indicated that there is a relationship between oxidative stress and PCOS. This might be a key mechanism for understanding PCOS disease. Therefore, oxidative stress might contribute to PCOS and its metabolic associations. Over all here we suggest that the PCOS may have an effect on the MDA level in blood, saliva and urine. It is possible, as suggested by Sheikhi *et al.* [43,44], that the increased salivary MDA level could occur through the mechanism of superoxide anion production during the oxidative stress that was seen in case of PCOS [13]. Also saliva and urine MDA levels were parallel increased with MDA level of blood in PCOS women. Measuring saliva and urine MDA level in the future in place of blood MDA level in PCOS women might be advantageous because of being none invasive biological samples and easy to collect them.

Furthermore, oxidative stress has been linked to insulin resistance as seen in this report and previous reports. Previous studies have emphasized that oxidative stress impairs glucose uptake in muscle and adipose tissue, and reduces insulin secretion from pancreatic beta cells [45]. Also some of study have showed that activation of stress-sensitive intracellular signaling pathways in both *in vitro* and *in vivo* results in insulin resistance and impaired insulin secretion [46–49]. In this work, we also reported that HOMA-IR values in PCOS women increased. These findings agreed with previous increased HOMA-IR values that were found in PCOS women [34,50]. Increased HOMA-IR values in PCOS women might be related with oxidative stress as reported previously in PCOS women that caused insulin resistance in PCOS women [51,52].

MaR1 is well recognized for playing a beneficial to living systems [53]. Previous *in vitro* and *in vivo* evidences have demonstrated that MaR1 exerts potent anti-inflammatory (mitigates LPS-induced acute lung injury and murine models of colitis) and proresolution activities under both physiologic and inflammatory disease conditions [54,55]. Therefore, in this study it was hypothesized that MaR1 might be associated with inflammation process in PCOS patients, which has not been explored before. In our study, we first time reported that blood, saliva and urine MaR1 concentrations were significantly decreased in PCOS subjects with compared with those in the control subjects. Correlation analysis demonstrated that blood, urine and saliva MaR1 concentrations were significantly correlated with parameters regarding, hormonal parameters, CRP, MDA, glucose metabolism, and insulin resistance. Hence, inflammation participates in the pathophysiology of PCOS as in in the physiologic process of many human inflammatory disorders [56]. Since MaR1 is compound that is capable of actively limit inflammation [57]. Here reported insufficiency MaR1 in PCOS patients might be associated with inflammation process in PCOS patients.

Also we first time here we analyzed salivary MaR1 level with and without PCOS. The level of MaR1 was validated in the saliva of study subjects, as previously described [32]. It was first time found that human MaR1 ELISA assay measure salivary MaR1 level as in blood or urine MaR1

concentration. The detection of urine salivary MaR1 level may provide additional advantages in elucidating the pathogenesis of PCOS as well as other inflammatory diseases (such as obesity, vasculitis, rheumatologic diseases, ulcerative colitis and asthma) because of collecting easily and not being invasive sample. Also this study has some the limitations: First of all, these studies are the small sample sizes. Second, our present data cannot explain whether decreased MaR1 is an initiator or end product. Third, this is a cross section study; the evaluated data were not obtained from patients with PCOS when they are "relatively" normal. Lastly, even though, this is first reports blood, saliva and urine MaR1 concentration in women with polycystic ovary syndrome, a well-designed multi-institutional longitudinal study might be needed to address this issue further before using as a new biological marker (MaR1) in case of PCOS.

5. Conclusions

In conclusion, in this prospective study of women with PCOS, we here identified a number of metabolic factors are associated with PCOS. These identified parameters represent some of the key metabolic abnormalities of PCOS. Unique findings of this study are that there is association of lower MaR1 levels and higher CRP and MDA with PCOS. Our study is the first to identify MaR1 may be a candidate marker and a risk factor and a significant predictor for PCOS. The clinical use of especially MaR1 in predicting women with PCOS warrants further studies to validate our first findings. Also, use of MaR1 in the future might be useful molecule to alleviate inflammatory disorders like PCOS disease. Since MaR1 is a macrophage mediator with effective proresolving and anti-inflammatory molecule that blocks the occurrence of various inflammatory conditions. Furthermore, measurements of MaR1 in saliva and urine are non-invasive, simple, and generally much preferred by patients, and thus may be a much acceptable alternative to plasma/serum sampling.

Author contributions

AY, SA conceived the study, designed the experiments, and performed the experiments. AY, SA, KU, ZKK, and DA analyzed data. AY and SA drafted the manuscript. All authors revised the manuscript and approved the final manuscript.

Ethics approval and consent to participate

The study protocol was accepted by the NOHU Non-Interventional Clinical Research Ethics Committee (Date: 2021, issue no: 12) and informed consent was taken from each participant subjects.

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Conflict of interest

The authors declare no conflict of interest.

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