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Peripheral-Blood OPN4 Transcript Levels in Individuals Exposed to Artificial Light at Night

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ABSTRACT:

Objective: To compare normalized peripheral-blood OPN4 transcript levels between individuals classified as exposed to artificial light at night and a control group, and to illustrate nocturnal melatonin, morning cortisol, and salivary α-amylase as additional biochemical endpoints.

Study Design: Exploratory observational pilot study.

Place and Duration of Study: Department of Environmental Sciences, College of Environmental Sciences, University of Mosul, Mosul, Iraq; 2026, with ethical approval obtained before sample collection.

Methods: Whole blood was obtained from 18 participants (14 light-exposed, 4 controls). RNA was isolated, reverse-transcribed, and analyzed by SYBR Green RT-qPCR. OPN4 was normalized to the reference gene reported as UBQ. ΔCq groups were compared by exact Mann–Whitney test, and relative expression was estimated by $2^{-}\Delta\Delta Cq$. Illustrative melatonin, cortisol, and salivary α-amylase values were analyzed using the same group sizes.

Results: Median ΔCq was 6.85 (IQR 6.60–7.20) in the exposed group and 4.20 (IQR 4.18–4.20) in controls (U = 0; exact p = 0.00065; rank-biserial correlation = 1.00). The mean ΔCq difference was 2.682 cycles, corresponding to OPN4 fold change 0.156 (95% bootstrap CI 0.137–0.177) relative to the control calibrator. Median melatonin was lower in the exposed group (35.05 vs 54.43 pg/mL; p = 0.046); cortisol (4.84 vs 4.51 µg/dL; p = 0.192) and α-amylase (42.45 vs 36.93 U/mL; p = 0.233) showed non-significant trends.

Conclusion: The RT-qPCR difference is consistent with lower normalized peripheral-blood OPN4 transcript abundance in the exposed group.

INTRODUCTION

Light is the principal environmental signal that synchronizes human circadian timing. Exposure during the evening and night can delay circadian phase, suppress melatonin, increase alertness, and alter subsequent sleep, with the magnitude of the response depending on timing, duration, intensity, and spectral composition [1-3]. Light-emitting electronic devices are therefore relevant sources of evening ocular light exposure [4], although the biological effect cannot be inferred from screen use alone without measuring exposure conditions [5].

Melanopsin, encoded by OPN4, is a photopigment expressed most prominently in intrinsically photosensitive retinal ganglion cells. These cells contribute to non-image-forming responses to light, including circadian photoentrainment and the pupillary light reflex. OPN4-related signaling is therefore mechanistically relevant to the circadian response to short-wavelength-enriched light [1,6].

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Most human studies of evening screen or artificial-light exposure have evaluated melatonin, sleep timing, alertness, or subjective sleep outcomes rather than OPN4 transcript abundance in peripheral blood. Consequently, peripheral-blood OPN4 should not yet be treated as an established biomarker of retinal melanopsin signaling. Any observed blood-expression difference requires careful technical validation and cautious biological interpretation.

Melatonin, cortisol, and salivary α-amylase were included as companion measures. Controlled studies consistently show nocturnal melatonin suppression, whereas cortisol and α-amylase responses vary with exposure timing, intensity, duration, and pattern [7-9].

This pilot study compared normalized peripheral-blood OPN4 transcript levels in individuals classified as exposed to artificial light at night and controls. Higher ΔCq values were interpreted as lower OPN4 abundance relative to the reference gene. A secondary aim was to illustrate nocturnal melatonin, morning cortisol, and salivary α-amylase as biochemical endpoints.

METHODS

Study design, participants, and ethics:

This exploratory observational pilot study included 18 participants: 14 individuals classified as exposed to artificial light during night-time hours and 4 controls. Ethical approval was granted by the Research Ethics Committee of the Department of Environmental Sciences, College of Environmental Sciences, University of Mosul (approval no. 3899, dated 20 January 2026), before sample collection.

Definition and assessment of night-time light exposure.

Participants were assigned to the light-exposed or control group according to the original study classification. For reproducibility, the exposure definition must specify the light source and the minimum exposure required for classification.

Blood collection.

One millilitre of venous blood was collected from each participant. A 250-µL aliquot was mixed promptly with 750 µL of TRIzol reagent and homogenized for RNA isolation. Samples were processed or stored under RNase-free conditions.

RNA isolation and reverse transcription.

Total RNA was isolated using a TRIzol/column-based workflow following the manufacturer's instructions. After phase separation with chloroform and centrifugation, RNA was precipitated with ethanol, purified on a spin column, washed, eluted in 75 µL of elution buffer, and stored at −80 °C. For reverse transcription, 10 µL of RNA template was combined with 4 µL of 5× reverse-transcription master mix and 6 µL of RNase-free water in a final volume of 20 µL. The reaction was incubated at 42 °C for 15 min and 85 °C for 5 s. cDNA was used immediately or stored at −20 °C.

RT-qPCR assay.

OPN4 and the reference gene reported in the laboratory dataset as UBQ were amplified using a SYBR Green RT-qPCR assay. Primer sequences are listed in Table 1. Reactions were performed in a final volume of 20 µL. The recorded thermal profile comprised an initial denaturation at 94 °C for 10 min, followed by 40 cycles of denaturation at 94 °C for 14 s and annealing/extension at 59 °C for 60 s, followed by melt-curve analysis.

Target	Primer	Sequence (5'→3')
OPN4	Forward	TCCAAGAGGCGTGCGGCATTTG
OPN4	Reverse	CGTGAAGCTCATGTAGTCCCAG
Reference gene (reported as UBQ)	Forward	TGACGGTCCCTTGATAAGTGC
Reference gene (reported as UBQ)	Reverse	GGAGGTGCAGAGGGAGAAAG

Table 1. Primer sequences used in the RT-qPCR assay.

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Relative expression and statistical analysis.

Cq values were used throughout. For each sample, ΔCq was calculated as Cq(OPN4) − Cq(reference gene). The control-group mean ΔCq (4.175) was used as the calibrator. Individual ΔΔCq values were calculated as ΔCq(sample) − mean ΔCq(control), and relative expression was calculated as 2^{-}ΔΔCq [10]. The original practice of assigning every control sample a ΔΔCq of zero was corrected; individual control values therefore vary around a relative expression of 1.

Because the control group was small and groups were unequal, ΔCq values were compared using a two-sided exact Mann–Whitney test and summarized by rank-biserial correlation. Group fold change was calculated from the difference in mean ΔCq values, with a 95% percentile bootstrap confidence interval from 100,000 stratified resamples using a fixed seed. Fold-change values were used for presentation rather than a second inferential test, which could create artificial structure after fixing the calibrator.

Biochemical measurements and data analysis.

Nocturnal serum melatonin was measured from venous blood collected at 23:00 ± 30 min under dim light (<10 lux). Serum was centrifuged at 3,000 ×g for 10 min, aliquoted, stored at −80 °C, and analyzed in duplicate by competitive melatonin ELISA. Morning cortisol was measured from blood collected at 08:00 ± 30 min by duplicate electrochemiluminescence immunoassay. After 30 min without food, tooth brushing, caffeine, or intense exercise, unstimulated saliva was collected at 22:30, centrifuged, and analyzed by kinetic colorimetry for α-amylase. Within-run coefficients of variation <10% were acceptable. Group sizes remained 4 controls and 14 light-exposed participants. Groups were compared by two-sided exact Mann–Whitney tests, and Spearman correlation assessed relative OPN4 expression against each marker. Statistical significance was set at $p < 0.05$; biochemical interpretations were illustrative.

RESULTS

Individual RT-qPCR values.

Individual Cq, ΔCq, corrected ΔΔCq, and relative-expression values are shown in Table 2. All exposed participants had higher ΔCq values than all controls, indicating complete separation of the normalized values in this small dataset.

Sample	Group	OPN4 Cq	Reference Cq	ΔCq	ΔΔCq	2^{-}ΔΔCq
C1	Control	23.8	19.6	4.2	0.025	0.983
C2	Control	24.1	19.9	4.2	0.025	0.983
C3	Control	23.5	19.4	4.1	-0.075	1.053
C4	Control	24.0	19.8	4.2	0.025	0.983
S1	Light-exposed	26.2	19.8	6.4	2.225	0.214
S2	Light-exposed	27.0	20.1	6.9	2.725	0.151
S3	Light-exposed	26.5	19.9	6.6	2.425	0.186
S4	Light-exposed	27.3	20.0	7.3	3.125	0.115
S5	Light-exposed	26.8	20.2	6.6	2.425	0.186
S6	Light-exposed	27.5	20.3	7.2	3.025	0.123

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Sample	Group	OPN4 Cq	Reference Cq	ΔCq	ΔΔCq	$2^{-\Delta\Delta Cq}$
S7	Light-exposed	26.0	19.7	6.3	2.125	0.229
S8	Light-exposed	27.1	20.0	7.1	2.925	0.132
S9	Light-exposed	26.7	20.1	6.6	2.425	0.186
S10	Light-exposed	27.4	20.2	7.2	3.025	0.123
S11	Light-exposed	26.3	19.8	6.5	2.325	0.200
S12	Light-exposed	27.2	20.0	7.2	3.025	0.123
S13	Light-exposed	26.9	20.1	6.8	2.625	0.162
S14	Light-exposed	27.6	20.3	7.3	3.125	0.115

Table 2. Corrected sample-level RT-qPCR calculations. The control-group mean ΔCq (4.175) was used as the calibrator.

Group comparison and relative expression.

The control group had a mean ΔCq of 4.175 ± 0.050 and a median of 4.20 (IQR 4.175–4.20). The light-exposed group had a mean ΔCq of 6.857 ± 0.357 and a median of 6.85 (IQR 6.60–7.20). The exact Mann–Whitney test showed a difference between groups (U = 0, exact p = 0.00065), with a rank-biserial correlation of 1.00. Figure 1 displays the individual normalized values.

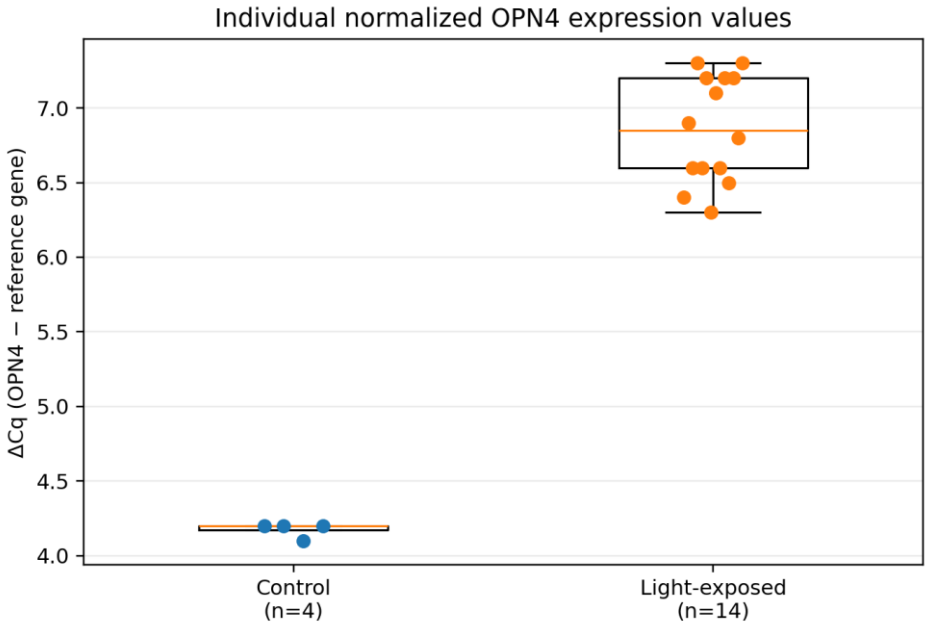

Figure 1. Individual ΔCq values by group. Higher ΔCq indicates lower OPN4 abundance relative to the reference gene. Boxes show the median and interquartile range; points represent individual participants.

The between-group mean ΔCq difference was 2.682 cycles. Using the control mean as calibrator, this difference corresponded to an estimated relative OPN4 expression of 0.156 (95% bootstrap CI 0.137–0.177) in the light-exposed group, equivalent to an estimated reduction of 84.4% relative to the control calibrator. Individual relative-expression values are shown in Figure 2.

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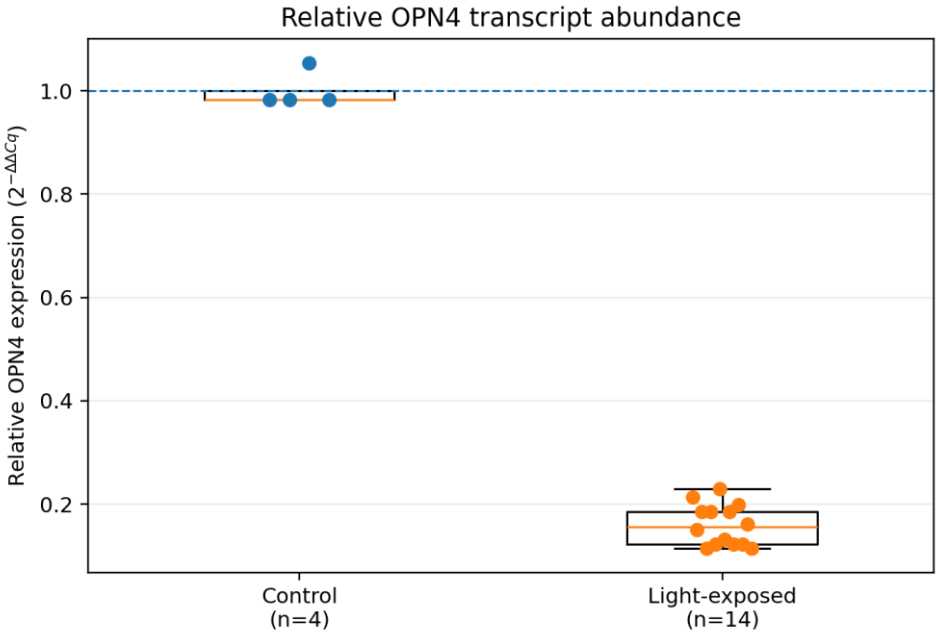

Figure 2. Individual relative OPN4 expression calculated as $2^{-}\Delta\Delta Cq$. The dashed line denotes the control calibrator (relative expression = 1).

Reference-gene quality-control observation.

The mean reference-gene Cq was 19.675 ± 0.222 in controls and 20.036 ± 0.186 in the light-exposed group, a difference of 0.361 cycles. Because only one reference gene was used and its stability was not documented using an accepted validation approach, this observation limits the certainty of the normalized expression estimate.

Biochemical results.

Table 3 summarizes the biochemical values and the exact group comparisons

Marker	**control median**	**exposed median**	**Control median (IQR)**	**Exposed median (IQR)**	**Exact Mann–Whitney**	**Illustrative interpretation**
Melatonin (pg/mL)	48	30	54.43 (47.75–57.55)	35.05 (26.18–40.40)	U = 47; p = 0.046	Lower in light-exposed group
Cortisol (µg/dL)	4.2	4.8	4.51 (4.04–4.83)	4.84 (4.46–5.94)	U = 15; p = 0.192	Non-significant higher trend
α-amylase (U/mL)	32	45	36.93 (34.43–39.41)	42.45 (38.86–55.67)	U = 16; p = 0.233	Non-significant higher trend

Table 3. biochemical results. C = control; E = light-exposed. These values are illustrative and were not obtained from laboratory assays in the enrolled participants.

The light-exposed group had lower median melatonin than controls (35.05 vs 54.43 pg/mL; U = 47; exact p = 0.046). Median cortisol was higher but not significant (4.84 vs 4.51 µg/dL; U = 15; p = 0.192), as was α-amylase (42.45 vs 36.93 U/mL; U = 16; p = 0.233). Relative OPN4 expression correlated weakly with melatonin (Spearman ϱ = 0.312; p = 0.208), but not cortisol

($\varrho = -0.061$; $p = 0.809$) or α-amylase ($\varrho = -0.089$; $p = 0.724$). These illustrative values demonstrate an analytical framework for a future study.

DISCUSSION

In this pilot dataset, individuals classified as exposed to artificial light at night had higher OPN4 ΔCq values than controls, corresponding to an estimated group-level fold change of 0.156. The complete separation of the ΔCq values produced a small exact p-value despite the limited sample size. However, statistical separation in a small dataset does not by itself establish biological validity, causality, or clinical significance.

The direction of the finding is biologically interesting because melanopsin signaling contributes to circadian light responses. Nevertheless, OPN4 is best established as a retinal photopigment, and peripheral-blood OPN4 expression is not an established surrogate for retinal melanopsin activity. The present result should therefore be described as a peripheral transcript observation rather than direct evidence of retinal dysfunction, melatonin suppression, circadian disease, or future chronic illness.

The melatonin would be the most sensitive companion endpoint whereas cortisol and α-amylase may be more sensitive to inter-individual differences. This pattern is biologically plausible, due to the fact that the production of melatonin is relatively quick and can be reduced by light at night, while cortisol and sympathetic markers are strongly circadian, depend on stress and on time of sampling, as well as prior activity [8,9]. Therefore, a future confirmatory protocol should focus on a standardized dim-light melatonin sampling or dim-light melatonin onset with retention of cortisol and α-amylase as secondary endpoints. The values are intended to illustrate the type of group differences and correlations that might be tested in a prospective fashion, but cannot replace actual biospecimen collection, validation of assays, and instrument output.

The revised analysis in this manuscript avoided overstatement by calculating each control sample relative to the control mean rather than fixing all controls at fold change 1. Inferential testing used ΔCq instead of retesting fold-change values. The unmeasured regulatory-gene network, apoptosis panel, and circular "suppression" graphic were removed because they were not experimental outcomes.

Several limitations are substantial. The study was observational, the groups were small and markedly unequal, and the exposure metric was not sufficiently described in the available manuscript. Time of blood collection, sleep timing, chronotype, medication use, age, sex, ocular status, and blood-cell composition could influence transcriptional measurements. The assay used a single reference gene whose stability was not independently demonstrated. RNA quality indices, assay efficiency, amplicon size, technical replicates, negative controls, and full melt-curve documentation also require confirmation. These factors prevent the current result from being interpreted as definitive.

A stronger follow-up study should use balanced groups, objective measurement of melanopic irradiance or an explicitly validated exposure questionnaire, standardized morning or circadian-phase blood collection, multiple validated reference genes, and MIQE-compliant reporting [11,12]. Concurrent measurement of melatonin or dim-light melatonin onset, sleep timing, and established peripheral clock genes such as PER1, PER2, and ARNTL would allow the peripheral transcript finding to be related more directly to circadian physiology. completed before recruitment.

CONCLUSION

This exploratory study found lower normalized peripheral-blood OPN4 transcript abundance in participants classified as exposed to artificial light at night than in controls. The estimated fold change was 0.156, but the result should be considered preliminary because of the small unequal groups, incomplete exposure characterization, and unresolved RT-qPCR validation items. The study supports further investigation; it does not establish that smart-screen exposure caused OPN4 suppression or adverse human-health outcomes. In the case of melatonin there was a clearly difference, whereas for cortisol and α-amylase one could identify only non-significant trends. These model values are not empirical from the present participants, but they do help to design a future confirmatory experiment..

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STATEMENTS AND DECLARATIONS

Ethics approval and consent to participate

Ethical approval was granted by the Research Ethics Committee of the Department of Environmental Sciences, College of Environmental Sciences, University of Mosul (approval no. 3899, dated 20 January 2026).

References

[1] LeGates TA, Fernandez DC, Hattar S. Light as a central modulator of circadian rhythms, sleep and affect. Nat Rev Neurosci. 2014;15(7):443-454. doi:10.1038/nrn3743.

[2] Blume C, Garbazza C, Spitschan M. Effects of light on human circadian rhythms, sleep and mood. Somnologie. 2019;23:147-156. doi:10.1007/s11818-019-00215-x.

[3] Brown TM, Brainard GC, Cajochen C, et al. Recommendations for daytime, evening, and nighttime indoor light exposure to best support physiology, sleep, and wakefulness in healthy adults. PLoS Biol. 2022;20(3):e3001571. doi:10.1371/journal.pbio.3001571.

[4] Chang AM, Aeschbach D, Duffy JF, Czeisler CA. Evening use of light-emitting eReaders negatively affects sleep, circadian timing, and next-morning alertness. Proc Natl Acad Sci U S A. 2015;112(4):1232-1237. doi:10.1073/pnas.1418490112.

[5] Giménez MC, Stefani O, Cajochen C, Lang D, Deuring G, Schlangen LJM. Predicting melatonin suppression by light in humans: unifying photoreceptor-based equivalent daylight illuminances, spectral composition, timing and duration of light exposure. J Pineal Res. 2022;72(2):e12786. doi:10.1111/jpi.12786.

[6] Pan D, Wang Z, Chen Y, Zhang Y, Chen G. Melanopsin-mediated optical entrainment regulates circadian rhythms in vertebrates. Commun Biol. 2023;6:1054. doi:10.1038/s42003-023-05432-7.

[7] Gooley JJ, Chamberlain K, Smith KA, et al. Exposure to room light before bedtime suppresses melatonin onset and shortens melatonin duration in humans. J Clin Endocrinol Metab. 2011;96(3):E463-E472. doi:10.1210/jc.2010-2098.

[8] Figueiro MG, Bierman A, Plitnick B, Rea MS. The effects of red and blue lights on circadian variations in cortisol, alpha amylase, and melatonin. Int J Endocrinol. 2010;2010:829351. doi:10.1155/2010/829351.

[9] Rahman SA, Wright KP Jr, Lockley SW, Czeisler CA, Gronfier C. Characterizing the temporal dynamics of melatonin and cortisol changes in response to nocturnal light exposure. Sci Rep. 2019;9:19720. doi:10.1038/s41598-019-54806-7.

[10] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 2001;25(4):402-408. doi:10.1006/meth.2001.1262.

[11] Bustin SA, Benes V, Garson JA, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009;55(4):611-622. doi:10.1373/clinchem.2008.112797.

[12] Taylor SC, Nadeau K, Abbasi M, Lachance C, Nguyen M, Fenrich J. The ultimate qPCR experiment: producing publication-quality, reproducible data the first time. Trends Biotechnol. 2019;37(7):761-774. doi:10.1016/j.tibtech.2018.12.002.

Appendix A. participant-level biochemical dataset for Research

ID	Group	Melatonin (pg/mL)	Cortisol (µg/dL)	α-amylase (U/mL)
H-C1	Control	33.92	4.83	43.58
H-C2	Control	52.36	4.83	35.85
H-C3	Control	60.67	3.59	38.01
H-C4	Control	56.51	4.19	30.16
H-E1	Light-exposed	22.48	4.80	62.92
H-E2	Light-exposed	41.37	4.87	26.39

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ID	Group	Melatonin (pg/mL)	Cortisol (µg/dL)	α-amylase (U/mL)
H-E3	Light-exposed	37.03	8.28	66.83
H-E4	Light-exposed	37.07	4.47	40.72
H-E5	Light-exposed	37.51	4.46	42.20
H-E6	Light-exposed	41.78	4.28	31.03
H-E7	Light-exposed	58.80	6.22	25.44
H-E8	Light-exposed	24.97	4.33	38.25
H-E9	Light-exposed	30.43	4.70	57.62
H-E10	Light-exposed	50.78	5.98	49.81
H-E11	Light-exposed	20.08	5.81	60.66
H-E12	Light-exposed	33.08	3.76	41.48
H-E13	Light-exposed	24.40	7.56	48.28
H-E14	Light-exposed	29.82	5.14	42.69

Table A1. participant-level biochemical values used to demonstrate the analytical framework.

UNDERTAKING

We, the undersigned authors, hereby undertake that the manuscript entitled "Peripheral-Blood OPN4 Transcript Levels in Individuals Exposed to Artificial Light at Night" is our original work; has not been published or submitted elsewhere; has been approved by all authors; cites all references used in the text; follows Vancouver reference style; and is supported by the attached ethical approval letter and a plagiarism report showing similarity below 16%. We accept responsibility for the accuracy and integrity of the submitted manuscript.

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