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Natural moisturising factor constituents in South African nursing students

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Abstract

Background: The majority of South African healthcare workers are Black Africans with dark-pigmented skin. Studies on how the markers of skin barrier function and natural moisturising factor (NMF) compare between dark and light-pigmented skin are limited. Quantifying NMF in a nursing student population during their practical training at university may provide valuable insight into their potential susceptibility to skin conditions associated with low NMF.

Objectives: The objectives of this study were to quantify and compare NMF content of Black African, Mixed Race and White nursing students from their dominant dorsal hand.

Methods: Forty-nine White, 32 Black African and 5 Mixed Race nursing students participated in this study. Tape strip samples were collected from the participants' dominant dorsal hand and NMF content was measured, including histidine (HIS), pyrrolidone carboxylic acid (PCA), trans-urocanic acid (t-UCA) and *cis*-urocanic acid (c-UCA), as well as cytokines interleukin-1 alpha (IL-1α) and interleukin-1 receptor antagonist (IL-1RA).

Results: No statistically significant differences in PCA, t-UCA, c-UCA, IL-1α or IL-1RA were found between Black African and White nursing students. HIS was significantly ($p = 0.001$) higher in White nursing students when compared to Black African students. The ratio of tot-UCA/HIS was significantly higher in Black Africans ($p = 0.0002$) when compared to White nursing students.

Conclusion: No significant differences were established in NMF content between White and Black African nursing students, other than HIS which was significantly higher in White students than in Black African students. Different HIS levels between the racial groups suggest variation in histidase activity which may be related to skin pH and pigmentation. This finding may suggest that nursing students at the beginning of their careers may have similar susceptibility to skin diseases related to NMF.

KEYWORDS

cis-urocanic acid, natural moisturising factor, pyrrolidone carboxylic acid, tertiary education, trans-urocanic acid

1 | INTRODUCTION

Natural moisturising factor (NMF) in human skin is one of the key components in maintaining skin barrier homeostasis. NMF mainly consists of free amino acids and their derivatives that act as humectants in the stratum corneum (SC) by attracting water molecules and hydrating the skin.[1] NMF components including urocanic acid (UCA) and pyrrolidone carboxylic acid (PCA) are products from the hydrolysis of the SC structural protein, filaggrin. Filaggrin is hydrolysed to arginine, glutamine and histidine (HIS), amongst others, during cornification above the stratum compactum, where a high quantity of water is still present. As the water content decreases in the outer layers of the SC, histidase is activated and HIS is deaminated to trans-urocanic acid (t-UCA), and PCA is produced from glutamine while arginine is converted to citrulline.[2,3] Finally, photoisomerisation of t-UCA to the c-UCA isomer takes place under ultraviolet-B (UV-B) radiation and serves as an endogenous sunscreen.[2] NMF components are hygroscopically active and by drawing water to the SC, they play a key role in maintaining SC hydration. Furthermore, t-UCA is a known acidifier of the SC and is therefore important for maintaining the pH gradient in the SC.[2–4]

Loss-of-function mutations in the filaggrin gene (*FLG*) have been strongly associated with the development of atopic dermatitis and have a strong correlation with the severity and onset of the disease.[3–5] This is due to the cascading effect that low filaggrin content has on the skin condition as it results in low NMF content, which causes low SC water content and increased skin surface pH.[6,7] The subsequent activation of serine proteases that are involved in degradation of corneodesmosomes and extracellular lipid processing enzymes may lead to compromised SC integrity.[3] Proinflammatory interleukin-1 (IL-1) cytokines play a significant role in the inflammatory response associated with atopic dermatitis,[8] and increased expression of IL-1 have been associated with *FLG* mutations.[9] Keratinocytes produce IL-1 alpha (IL-1α)[10] to initiate an inflammatory response, while IL-1 receptor antagonist (IL-1RA) antagonises the effects of IL-1α.

Furthermore, *FLG* null mutations and atopic dermatitis have been associated with an increased risk for developing hand eczema,[11,12] and chronic irritant contact dermatitis.[13] Persons with a genetic predisposition due to *FLG* mutations may therefore be more susceptible to developing contact dermatitis, which would be relevant for persons who are occupationally exposed to irritants, e.g., hairdressers, bakers, tile settlers, metal surface processors and healthcare workers.[14] Healthcare workers are required to follow a strict hand hygiene regimen to prevent nosocomial infections in healthcare settings.[15] This however increases their exposure to wet work and irritants such as water and hand soap, as well as skin occlusion which increases the probability of developing contact dermatitis.[16] Several studies showed that skin irritants reduce NMF levels in the skin.[17,18] Healthcare workers are especially vulnerable to these irritants, given that their occupational exposure to skin irritants may lead to a reduction in NMF levels. Furthermore, if these individuals have intrinsically lower NMF due to *FLG* mutations, the impact of these irritants could be significantly amplified, thereby increasing their vulnerability to dermatological conditions associated with low NMF levels.

A study comparing African and Caucasian skin in vitro found that filaggrin was more expressed and processed in the Caucasian epidermis than in the African epidermis. The study also suggested that the modulation of most enzyme genes differed between the two skin types, indicating that the production of NMF could vary between them.[19] PCA concentration was measured in South African albino Africans, Black Africans and Whites in 2016. PCA levels were higher in albino Africans than in Black Africans and Whites, while Black Africans had significantly higher PCA levels than Whites.[20] NMF content was also measured in South African amaXhosa patients with atopic dermatitis to investigate the relationship between atopic dermatitis, NMF content and *FLG* loss of function mutations. PCA and UCA levels were established to be significantly lower in patients with atopic dermatitis when compared to patients in the control group. However, these results were established in patients without a *FLG* mutation suggesting that there is an interplay between systemic T-helper 2 cell (Th-2) inflammation and *FLG* suppression in South African amaXhosa patients.[21] The majority of the nursing workforce in South Africa is Black African,[22] and NMF content studies on dark pigmented skin are limited to these two studies, which did not include a high-risk population such as nursing students.

This study aimed to quantify and compare NMF content of Black African and White nursing students. Investigating NMF content in nursing students during practical training may provide insight into the potential development of skin disease during their tertiary education.

2 | METHODS

This study was conducted in accordance with the Helsinki Declaration[23] and approved by the Health Research Ethics Committee of the North-West University, South Africa (Ethics approval number: NWU-00337-16-A1).

2.1 | Study population

Female nursing students from the North-West University, South Africa were recruited to participate in this study. For the purpose of this publication, the term race refers to groups with similar physical features and skin colour[24] where White is used for light-pigmented skin and Black African for dark-pigmented skin. Participants using oral contraceptives or retinoid medication and with a history of childhood eczema, rhinitis or asthma were excluded from the study. After obtaining written informed consent, 49 White, 32 Black African and 5 Indian / Mixed race nursing students (second to fourth-year undergraduate students) participated in this study. All of the participants were non-smokers and between the ages of 18 and 40 years.

The participants were grouped into self-reported racial groups and corresponding skin pigment types (Fitzpatrick scale)[25] as well as colour space values of the Commission International d'Eclairage (CIE) represented by L*a*b coordinates, as summarised in Table 1. The L* value indicates the lightness of the skin (0 = black and 100 = white), the a* value indicates the range between green and red (green <0 < red) and the b* value is the range between blue and yellow (blue <0 < yellow). These values were measured on the volar wrist of the non-dominant forearms[26] of the participants with a Colorimeter (CL-400) manufactured by Courage and Khazaka (CK) electronic GmBH, Germany.

2.2 | NMF collection and analyses

Sequential tape stripping was used to collect NMF from the upper layers of the participants' SC at the beginning of the academic year (January/February), 2018. Participants were asked not to wash their hands or apply any cosmetic products to their hands 12 h prior to the NMF sample collection.

Six tape strips were applied to the same marked skin area on the dominant dorsal hand of the participants with standardised force (10 N) using a disc pressure applicator (Cuderm, Texas). The first three tape strips were discarded to correct for any residual cosmetic products still present on the skin and the remaining samples were stored at −80°C until further analysis. Tape strips 4 and 5 were combined for NMF component analyses, and tape strip 6 was used for the cytokine analyses. The total SC protein content on the tape strips was measured by determining the optical density at 850 nm with the D-Squame Scan 850A (Monaderm, Monaco). NMF components HIS, PCA, t-UCA, c-UCA and total NMF (tot-NMF which is the sum of individual components) were determined by high-performance liquid chromatography after extraction with water as described elsewhere.[27] The values were normalised according to the total amount of SC proteins determined with the optical density measurements of the tape strip and expressed as mmol per gram of proteins.[28] The analyses of cytokines IL-1α and IL-1RA on the tape strips were performed by Mesoscale multiplex immunoassay (MESO QuickPlex SQ 120 assay, MSD, Rockville, Maryland). The cytokine concentrations were normalised for protein amount determined with Pierce Protein Assays (Thermo Fischer Scientific).

The metabolism of HIS to t-UCA is expressed as a ratio tot-UCA/HIS, and the conversion of t-UCA to c-UCA is expressed as the ratio of c-UCA/tot-UCA.

TABLE 1 Participant skin colour classifications according to self-reported race, L*a*b colour space value (L*) and skin pigment types.

Self-reported race	*N*	L* value range	Skin pigment type[25]
White	49	58.68–70.46	II–III
Indian/Mixed race	5	47.79–59.59	III–IV
Black African	32	40.88–55.99	IV–VI

2.3 | Statistical analyses

Statistical analyses were performed with Statistica 64 version 13.3 (Statsoft Inc. Palo Alto), and figures were generated with GraphPad Prism® version 7 (GraphPad Software).

A normal distribution of the data was assumed according to the central limit theorem,[29] as the sample sizes used in parametric tests were above 30 and the constant variance of the data was tested with Levene's test and confirmed ($p > 0.05$). Statistical outliers were detected with a Grubbs' test[30] and a total of four outliers were removed from the data before the analyses. Descriptive results are presented as geometric means (GM) and confidence intervals (CI: 95%). One-way analysis of variance (ANOVA) was used to determine if NMF constituents significantly differed with age and independent t-tests for differences in hand dominance (Right or Left). Statistical analyses were not performed on Mixed Race student data, due to the small number of students that participated in the study; however, descriptive statistics are reported.

Differences in NMF constituents between the racial groups were tested with independent t-tests (significant if $p \leq 0.05$). Effect sizes were calculated as Cohen's *d* test statistic [$(Mean_{(group\ 2)} - Mean_{(group\ 1)})/pooled\ standard\ deviation$] and interpreted as follows: small effect $d = 0.2$; moderate effect $d = 0.5$ and large effect $d = 0.8$.[31] Pearson correlations were used to test for significant correlations between the NMF constituents and the relationship between NMF constituents is displayed in linear regression graphs. Correlations were significant if $p \leq 0.05$, and the strength of the relationship was categorized as weak if $r = 0.00$–0.39; moderate if $r = 0.40$–0.59; and strong if $r = 0.60$–1.0.[32]

3 | RESULTS

NMF tape strip samples were collected from female nursing students (mean age 20 ± 2.42) at the beginning of the academic year. NMF tape strip samples were collected from the dominant dorsal hand of participants, and the influence of hand dominance (Right or Left) had no significant influence ($p > 0.05$) on the results. Participant age also had no significant influence on the results ($p > 0.05$).

NMF components and cytokine results are presented in Table 2. The geometric means of total protein content for White nursing students was 79.48 μg/mL (CI 37.72, 138.94) and 65.34 μg/mL (CI 26.04, 155.09) for Black African nursing students, while Mixed Race students' total protein content was 87.50 μg/mL (CI 67.42, 134.33). The total NMF (tot-NMF) of White nursing students was 0.65 mmol/g protein (CI 0.25, 1.18) and 0.55 mmol/g protein (CI 0.28, 1.02) for Black African nursing students, while Mixed Race students' tot-NMF was 0.48; (CI 0.17, 1.00). Levels of NMF components ranged between 0.004 and 1.37 mmol/g protein, with t-UCA and c-UCA both with the lowest concentrations (0.004 mmol/g protein) and PCA with the highest concentration (1.37 mmol/g protein) in all the racial groups. GM IL-1α in all the racial groups was 0.03 ng/μg protein (CI 0.01, 0.10), while GM IL-1RA ranged from 0.14 ng/μg protein (White students) to 0.21 ng/μg protein (Mixed Race students).

TABLE 2 NMF constituents (mmol/g protein) and cytokines (ng/μg protein) of White, Black African and Mixed Race nursing students ($N = 84$).

	White ($n = 47$)		Black Africans ($n = 32$)		Mixed Race ($n = 5$)	
NMF Constituents (mmol/g protein)	**GM**	**CI 95%**	**GM**	**CI 95%**	**GM**	**CI 95%**
Tot-protein (μg/mL)	79.48	37.72, 138.94	65.34	26.04, 155.09	87.50	67.42, 134.33
HIS	**0.15**[a]	0.04, 0.32	**0.09**[a]	0.04, 0.24	0.10	0.04, 0.24
t-UCA	0.03	0.01, 0.06	0.03	0.02, 0.07	0.02	0.01, 0.05
c-UCA	0.05	0.01, 0.13	0.05	0.03, 0.09	0.03	0.01, 0.08
tot-UCA	0.08	0.02, 0.19	0.08	0.04, 0.15	0.06	0.02, 0.12
PCA	0.41	0.17, 0.77	0.38	0.19, 0.76	0.31	0.11, 0.64
tot-NMF	0.65	0.25, 1.18	0.55	0.28, 1.02	0.48	0.17, 1.00
tot-UCA/HIS	**0.56**[b]	0.20, 1.37	**0.91**[b]	0.31, 2.16	0.56	0.39, 1.22
c-UCA/tot-UCA	**0.64**[c]	0.57, 0.69	**0.60**[c]	0.51, 0.66	0.58	0.48, 0.66
Cytokines (ng/μg protein)						
IL-1α	0.03	0.01, 0.09	0.03	0.01, 0.10	0.03	0.01, 0.06
IL-1RA	0.14	0.04, 0.42	0.16	0.03, 0.35	0.21	0.05, 0.15

Note: [a–d]Significant differences ($p = 0.001$) between racial groups in NMF constituents.

Abbreviations: c-UCA, cis-urocanic acid; c-UCA/tot-UCA, cis-urocanic acid per total urocanic acid; HIS, histidine; IL-1RA, interleukin-1 receptor antagonist; IL-1α, interleukin-1 alpha; PCA, pyrrolidone carboxylic acid; tot-NMF, total natural moisturising factor; Tot-Protein, Total protein content; tot-UCA, total urocanic acid; tot-UCA/HIS, total urocanic acid per histidine; t-UCA, trans-urocanic acid.

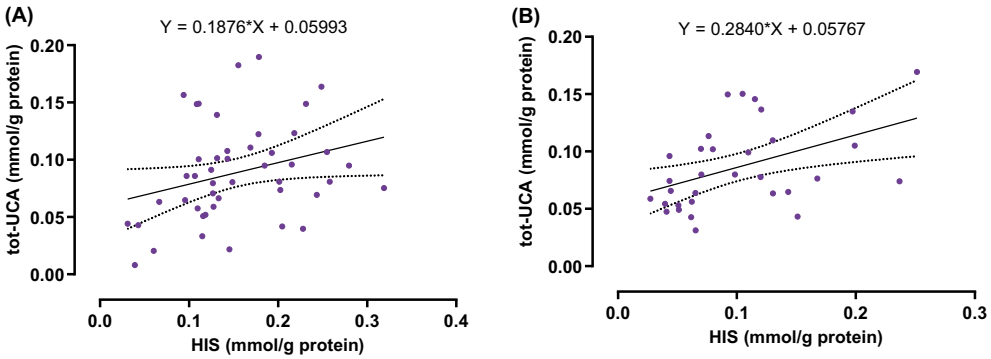

FIGURE 1 Linear regression graphs of HIS (mmol/g protein) and tot-UCA (mmol/g protein) in White (A) and Black African (B) nursing students with 95% Confidence Intervals (dotted lines).

No significant differences ($p > 0.05$) were established in total protein content, t-UCA, c-UCA, PCA, tot-NMF or in the cytokines IL-1α and IL-1RA between Black African and White nursing students. A significant difference was, however, found in HIS concentration. HIS concentration was significantly lower ($p = 0.001$) in Black African nursing students (GM 0.09; CI 0.04, 0.24) when compared to White nursing students (GM 0.15; CI 0.04, 0.32) with a large effect size of 0.84.

Significant strong positive correlations exist between t-UCA and c-UCA in both racial groups (Whites $r = 0.99$, $p \leq 0.05$; Black Africans $r = 0.97$, $p = 0.001$). The metabolism of HIS to UCA was assessed with t-UCA and c-UCA combined as total-UCA (tot-UCA) per HIS. Linear regression graphs were used to illustrate this relationship between HIS and tot-UCA in White nursing students (Figure 1A) and Black African nursing students (Figure 1B). Correlations between HIS and tot-UCA were significant ($p = 0.001$) in both racial groups, with a weak positive correlation in White students ($r = 0.29$) and a moderate positive correlation in Black African students ($r = 0.46$).

Although no significant differences were established in tot-UCA between the racial groups, a difference in the pathway of HIS to UCA is however present, as the ratio calculated between these metabolites (tot-UCA/HIS) differed significantly between the racial groups. The ratio of tot-UCA/HIS was significantly lower ($p = 0.0002$) in White students (GM ratio 0.56, CI 0.20, 1.37) than in Black African students (GM ratio 0.91, CI 0.31, 2.16) with a moderate effect size of 0.45. However, the ratio of c-UCA/tot-UCA was significantly higher ($p = 0.0001$) in White students (ratio GM 0.64, CI 0.57, 0.69) when compared to Black African students (ratio GM 0.60, CI 0.51, 0.66) with a moderate effect size of 0.52.

4 | DISCUSSION

As part of their training nursing students are required to complete practical training in hospitals, where they are subjected to a high amount of wet work including frequent hand washing, which may

increase their risk of developing contact dermatitis. Wet work and frequent hand washing are known to decrease NMF, which may lead to a compromised skin barrier and skin irritation.[17] This may be due to Sodium Lauryl Sulphate (SLS) that causes denaturation of the proteins in the cornified envelope, which may cause leakage of NMF components from the corneocytes.[33] If an individual with intrinsically lower NMF levels, e.g., due to skin phototype or *FLG* mutations might increase their susceptibility for contact dermatitis. Therefore, measuring NMF may aid in determining if these students are susceptible to skin diseases associated with low NMF levels. NMF components that were measured included HIS, t-UCA, c-UCA, PCA and their sum was expressed as total NMF (tot-NMF). Furthermore, total protein content, and cytokines (IL-1α and IL-1RA) were also quantified. The ratio of tot-UCA/HIS was calculated to illustrate the metabolism of HIS to UCA by the enzyme histidase and the c-UCA/tot-UCA ratio to illustrate t-UCA conversion to c-UCA by UV-B radiation.[2,3]

Only five Mixed Race students participated in this study, and were, therefore, not included in the statistical comparison analyses, but descriptive statistics were reported. No significant differences were established for tot-NMF, t-UCA, c-UCA and PCA, between Black African and White nursing students. These results are contradictory to the findings of a previous study which found a significant difference in PCA concentrations between Black African and Whites in a South African population.[20] These conflicting results may be due to anatomical variation in the sampling sites as the present study collected tape strips from the dorsal hands of participants, while Raj et al.[20] collected tape strip samples from the cheeks of participants. Another possible explanation may be that there are age-related differences in NMF levels between the present study and the study by Raj et al.[20] as the mean age in this study was 20 ± 2.42 years compared to 41 ± 2.77 years in the study conducted by Raj et al.[20] Recently Howard et al.[34] established that NMF content increases with age in healthy subjects. Isomerisation of t-UCA to c-UCA has been established to be influenced by skin pigmentation.[35] This is due to a higher degree of backscattering of UV rays in light-pigmented skin, as fewer UV rays are absorbed due to the lower melanin content present. However, at higher doses of UV radiation a dynamic equilibrium is reached between t-UCA and c-UCA.[36] In our study c-UCA amounted to 64% and 60%, respectively for White and Black African participants, which is within the photostationary phase (60%–65%). Consequently, no significant difference was established in c-UCA between White and Black African participants in our study. No differences in c-UCA between different skin phototypes after multiple UV exposures were also reported in a study by Krien et al.[36]

We found, however, that HIS was significantly ($p = 0.001$) lower in Black African students when compared to White students. HIS is one of the filaggrin degradation products, which is deaminated by the enzyme histidase to form t-UCA.[2] The significantly higher ratio of tot-UCA per HIS found in Black African students, suggests that a proportion of HIS metabolised to t-UCA is higher in Black African students which might explain their lower HIS levels. This difference in HIS conversion to tot-UCA may be due to the activity of histidase that differs between the two groups.[19]

Histidase functions optimally at an acidic pH and the dendrites of melanocytes in dark-pigmented skin (IV-V) have been found to be more acidic than those in lightly pigmented skin (I-II) and contributed to a higher activity of histidase in dark-pigmented skin.[37] Higher activity of histidase may therefore increase the production of t-UCA from HIS.[3]

Similar t-UCA levels in both racial groups suggest that skin surface pH should be similar in both racial groups, because t-UCA is a known acidifier of the SC.[2–4] Skin surface pH of the participants of the present study has been investigated previously, and Black African nursing students' skin surface pH significantly higher than White nursing students (Skin surface pH of 5.35 for Black African nursing students, and 4.73 for White nursing students, as measured on the volar forearm).[38] Higher skin surface pH of Black African nursing students, may support the hypothesis that personal habits contribute to the higher skin surface pH in this group. The type of cosmetic products the students use at home may play a role in their maintaining their skin surface pH. In our larger study it was determined from participant questionnaires that Black African nursing students used petrolatum for dry skin which may have influenced the skin surface pH in this group. Another reason might be the difference in the amount of other skin components that are known to influence skin pH, such as sphingoid bases and free fatty acids.[39,40]

Similar NMF content in racial groups may suggest a similar susceptibility to skin diseases associated with NMF content. Atopic dermatitis prevalence in the United States was determined to be higher in African American participants when compared to European Americans, even though *FLG* loss-of-function mutations were six times less common in African Americans.[41] However, in a study that tested irritant contact dermatitis susceptibility between African Americans and Caucasians, the threshold for irritancy was significantly higher in African Americans which indicated that Caucasians were more susceptible to irritant contact dermatitis.[42] The literature on the prevalence of skin diseases between different racial groups is contradicting and the susceptibility to skin diseases is determined by numerous factors, of which NMF is only one. The results of this study have shown that there is no significant difference in NMF content between the two racial groups this early in the nursing students' careers. If only considering the role of NMF in the susceptibility to skin diseases, the results indicated similar NMF content, and therefore a similar susceptibility. However, a person's susceptibility to skin diseases is determined by various factors. A study investigating hand eczema prevalence in metalwork apprentices established associations between hand eczema and *FLG* mutations but not between hand eczema and NMF content.[43]

Future studies may investigate the influence of different types of cosmetic products on NMF content in participants. More research on the anatomical variation of NMF content is also necessary as occupational skin diseases such as contact dermatitis often occur on the anatomical site most exposed e.g. the hands.

One limitation of this study was that NMF content could only be measured at a single anatomical region and not on the cheeks of

participants which prevented accurate comparison of the findings of this study to published research. NMF was measured on the dorsal aspect of participants' hands to quantify NMF at the site most exposed to frequent hand cleansing and wet work in a nursing student population. NMF was measured on participants' hands for this data to be used in a larger study to determine the susceptibility of nursing students to occupational skin diseases.

5 | CONCLUSION

NMF content in this study generally did not differ between the two racial groups, as the only significant difference was established in the HIS concentration and HIS to tot-UCA metabolism. An increased ratio between HIS and its metabolite UCA in Black African nursing students may indicate higher activity of histidase in this group. However, tot-UCA content did not differ significantly between the two racial groups. The observation of similar Natural Moisturising Factor (NMF) content between the racial groups suggests similar hygroscopic properties between the groups. This may indicate that the intrinsic susceptibility to skin diseases related to NMF content does not differ significantly between these groups this early in their careers. However, this study also illustrated that more research is necessary on NMF content comparisons between racial groups as the findings of this study contradicted previous studies. More research would be necessary before interventions based on NMF content differences between dark- and light-pigmented skin can be recommended. This study contributed valuable data on NMF quantity in a high-risk group and the comparison of NMF between two racial groups in South Africa.

AUTHOR CONTRIBUTIONS

Anja Franken: Conceptualization; supervision; writing – review and editing; investigation; methodology; funding acquisition. **Monica Young:** Investigation; writing – original draft; writing – review and editing; methodology. **Johannes Lodewykus du Plessis:** Conceptualization; writing – review and editing; supervision; investigation. **Sanja Kezic:** Methodology; writing – review and editing. **Ivone Jakasa:** Methodology; writing – review and editing; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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