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Stability and Expression Levels of miR-130b, miR-138 and miR-155 for Age and Gender Prediction in Igbo and Yoruba Populations of Nigeria

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Abstract

Age estimation remains a major challenge in forensic investigations, particularly when biological evidence lacks contextual information. MicroRNAs (miRNAs) have emerged as promising molecular markers due to their tissue specificity and relative stability. This study investigated the stability and expression patterns of selected skin-specific miRNAs (miR-130b, miR-138 and miR-155) for age and gender prediction among individuals from Igbo and Yoruba ethnic groups in Nigeria. Skin swab samples were collected from fifty healthy adult volunteers (25 Igbo and 25 Yoruba), aged 20–40 years. Total RNA was extracted using the TRIzol® protocol, followed by spectrophotometric assessment of RNA purity and concentration. Complementary DNA synthesis and quantitative polymerase chain reaction (qPCR) were performed using LunaScript RT Supermix and Luna Universal qPCR Master Mix, respectively. GAPDH and ACTB were used as reference genes. Statistical analyses were conducted using IBM SPSS (version 26), including descriptive statistics, Pearson correlation, analysis of variance and linear regression. RNA purity values were generally within acceptable limits across all samples. miR-130b showed stable expression across age groups, genders and ethnicities, whereas miR-138 and miR-155 exhibited variable expression patterns. Linear regression analysis revealed a significant association between miR-130b expression and age in Yoruba females ($p<0.05$). Additionally, miR-155 and miR-138 showed significant associations with age across all genders ($p<0.05$). The findings suggest that miR-130b is a stable skin biomarker suitable for forensic identification of skin swab samples, while miR-155 and miR-138 demonstrate potential for age and gender prediction. Further studies with larger population sizes are recommended to validate these biomarkers for forensic applications.

Keywords: MicroRNA; Forensic age estimation; Skin swab; Gender prediction; Nigerian population; miR-130b; miR-138; miR-155

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INTRODUCTION

Ageing is driven by a combination of intrinsic and extrinsic factors that influence cellular integrity and tissue homeostasis over time. At the molecular level, ageing is characterised by genomic instability, telomere attrition, epigenetic alterations, mitochondrial dysfunction, loss of proteostasis and cellular senescence (López-Otín et al., 2013; Meiners et al., 2015). These processes collectively contribute to functional decline and phenotypic changes observed in ageing tissues. Recent studies have demonstrated that RNA degradation follows predictable time-dependent patterns influenced by environmental exposure, making RNA a promising molecular marker for forensic estimation of bloodstain age (Sirker et al., 2019; Hanson & Ballantyne, 2020; Anderson et al., 2020). In human populations, ageing becomes more biologically and clinically apparent between the ages of 40 and 60 years, a period often associated with the onset of age-related diseases (Amarya et al., 2018). However, molecular ageing processes begin much earlier and progress gradually, affecting all organs of the body (Ortega, 2014; Ludwig et al., 2016). These molecular changes have motivated the search for reliable biological markers that can be used to estimate age objectively, particularly in forensic contexts. The skin is one of the most accessible and visibly affected organs during ageing. It undergoes progressive structural and functional changes, including thinning of the epidermis, reduced elasticity, delayed wound healing, increased fragility and pigmentation abnormalities (Zouboulis & Makrantonaki, 2011; Mancini et al., 2014). Age-related skin changes arise from both intrinsic factors such as genetic programming and cellular senescence, and extrinsic influences such as ultraviolet radiation and environmental exposure (Zhang & Duan, 2018; D'Arino et al., 2023). These characteristics make skin a valuable tissue for forensic investigations, especially when other biological samples are unavailable or degraded. Intrinsic skin ageing reflects chronological tissue atrophy and reduced regenerative capacity, whereas extrinsic ageing accelerates these changes through environmental stressors, particularly sunlight (Lee et al., 2021; Shin et al., 2023). At the cellular level, skin ageing is associated with altered gene regulation, senescent fibroblasts and keratinocytes, and changes in regulatory RNA molecules (Gerasymchuk et al., 2020). These molecular alterations provide an opportunity to explore nucleic acid-based biomarkers for forensic age estimation.

MicroRNAs (miRNAs) are short, non-coding RNA molecules that regulate gene expression post-transcriptionally by promoting mRNA degradation or inhibiting translation (O'Brien et al., 2018). They are known for their tissue-specific expression patterns and remarkable stability, even under adverse environmental conditions. These properties have made miRNAs attractive candidates for forensic applications, including tissue identification, body fluid differentiation and age estimation (Mancini et al., 2014; Iroanya et al., 2024). Emerging evidence indicates that miRNA expression profiles change with age and differ between males and females, reflecting sex-specific biological regulation (Dai & Ahmed, 2014; Lin et al., 2022). In skin tissue, several miRNAs have been linked to keratinocyte and fibroblast senescence, suggesting their potential utility as age-related biomarkers (Rivetti di Val Cervo et al., 2012; Gerasymchuk et al., 2020). Among these, miR-130b, miR-138 and miR-155 have been reported to play key roles in cellular senescence, tissue ageing and gene regulatory networks relevant to skin biology. Within forensic science, the ability to estimate age and gender from trace biological samples such as skin swabs would significantly enhance investigative outcomes. Despite advances in forensic molecular biology, population-specific data on miRNA expression patterns remain limited, particularly in African populations. Genetic diversity, environmental factors and lifestyle differences can influence gene expression, underscoring the need for population-based studies (Ludwig et al., 2016). Therefore, this study investigated the stability and expression levels of selected skin-specific miRNAs (miR-130b, miR-138 and miR-155) for age and gender prediction among individuals from Igbo and Yoruba ethnic groups in Nigeria. By examining miRNA expression patterns across age groups, genders and ethnicities, this study aims to contribute population-relevant molecular data to forensic age estimation research. Advances in forensic molecular biology have further highlighted the utility of messenger RNA and other RNA species for post-depositional timing of biological evidence (Zapico & Ubelaker, 2020; Bauer & Patzelt, 2021).

Materials and methods

Study population and sample size

This study was conducted using a voluntary sampling approach. A total of fifty (50) healthy adult Nigerian participants were recruited, comprising twenty-five (25) individuals from the Igbo ethnic group and twenty-five (25) individuals from the Yoruba ethnic group. The participants included both males and females and were within the age range of 20 to 40 years. Individuals were grouped according to age and gender for subsequent analysis.

Ethical approval and informed consent

Ethical approval for this study was obtained from the Health Research Ethics Committee of the University of Lagos, Nigeria **(Approval number: CMUL/HREC/03/22/1039).** The purpose and procedures of the study were clearly explained to all participants prior to sample collection. Written informed consent was obtained from each participant before inclusion in the study, in accordance with ethical guidelines for research involving human subjects.

Collection and processing of skin swab samples

Skin swab samples were collected using sterile, labelled swab sticks. The swabs were gently rubbed on the skin surface of each participant to obtain epidermal cells. The tips of the swab sticks containing the skin samples were immediately broken into labelled 2 mL microcentrifuge tubes containing 1 mL of TRIzol® reagent. The tubes were placed on ice and transported to the Chevron Molecular Biology Laboratory, Lagos University Teaching Hospital. Samples were stored at −80 °C prior to molecular analysis.

Total ribonucleic acid (RNA) extraction

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Total RNA was extracted from the skin swab samples using the TRIzol® extraction protocol as described by Tu et al. (2018). The extraction procedure consisted of homogenisation, phase separation, RNA precipitation and washing. Briefly, 0.2 mL of chloroform was added to each tube containing 1 mL of TRIzol® reagent and the skin swab. The mixture was vortexed for 10–15 seconds and centrifuged at 12,000 rpm for 15 minutes. The aqueous phase was carefully transferred into new microcentrifuge tubes, after which 0.5 mL of isopropanol was added to precipitate the RNA. The samples were incubated at −80 °C for 30 minutes and centrifuged at 12,000 rpm for 10 minutes. The RNA pellets obtained were washed with 75% ethanol, centrifuged at 12,000 rpm for 5 minutes and air-dried. The pellets were dissolved in 50 µL of nuclease-free water and stored at −80 °C until further analysis.

Spectrophotometric analysis of RNA purity and concentration

The purity and concentration of extracted RNA samples were determined using a NanoDrop 1000 spectrophotometer (Thermo Scientific). Absorbance ratios at A260/280 and A260/230 were recorded to assess RNA purity, while RNA concentration was expressed in ng/µL.

Selection of primers

Primers for the target microRNAs (miR-130b, miR-138 and miR-155) and reference genes (GAPDH and ACTB) were selected based on previously published literature (Mancini et al., 2014). Primer specificity and quality were validated using NetPrimer and OligoAnalyzer tools. The selected miRNAs are associated with keratinocyte and fibroblast senescence.

Table 1: Primer sequences used for quantitative polymerase chain reaction (qPCR)

GENES	PRIMER SEQUENCES
miR-130b-3p	Forward 5′-CTGGTAGGGTACAGTACTGTGATA-3′ Reverse 5′-CTGGTGTCGTGGAGTCGGC-3′
miR-138	Forward5′-TCCGAGCCTGACTAAGTGTTGTGGTCGA-3′ Reverse 5′-GTGCAGGGTCCGAGGT-3′
miR-155	Forward 5′-GTTAATGCTAATTGTGATAGGGG-3′ Reverse 5′-CATCATACCCTGTTAATGCTAAC-3′
GAPDH	Forward 5′-GTCTCCTCTGACTTCAACAGCG-3′ Reverse 5′-ACCACCCTGTTGCTGTAGCCAA-3′
ACTB	Forward 5’- GACCTGTACGCCAACACAGT - 3’ Reverse 5’ -CTCCTTCTGCATCCTGTCGG - 3’

Source: Mancini *et al*. (2014)

Complementary deoxyribonucleic acid (cDNA) synthesis

Complementary DNA (cDNA) synthesis was performed using the LunaScript RT Supermix cDNA synthesis kit following the manufacturer’s instructions. Each reaction mixture consisted of 4 µL of template RNA, 4 µL of LunaScript RT Supermix (5×) and 12 µL of nuclease-free water, giving a total reaction volume of 20 µL. The thermal cycling conditions were set at 25 °C for 2 minutes (primer annealing), 55 °C for 10 minutes (cDNA synthesis) and 95 °C for 1 minute (enzyme inactivation).

Quantitative polymerase chain reaction (qPCR)

Quantitative polymerase chain reaction was carried out using Luna Universal qPCR Master Mix. Each reaction mixture contained 10 µL of master mix, 0.5 µL each of forward and reverse primers, 5 µL of cDNA template and 4 µL of nuclease-free water. The amplification protocol included an initial denaturation at 95 °C for 60 seconds, followed by 40 cycles of denaturation at 95 °C for 15 seconds and extension at 60 °C for 20 seconds. Melt curve analysis was performed to confirm product specificity.

Statistical analysis

Quantitative PCR data, spectrophotometric measurements and demographic variables were analysed using IBM SPSS Statistics version 26. Descriptive statistics were used to summarise RNA purity, concentration and Cq values. Inferential analyses included Pearson correlation, analysis of variance (ANOVA) and linear regression to assess relationships between miRNA expression, age, gender and ethnicity. Statistical significance was set at $p \leq 0.05$. Graphs were generated using Microsoft Excel.

RESULTS

Descriptive statistics of RNA purity and concentration from skin swab samples

Spectrophotometric analysis revealed that RNA purity values for the majority of samples fell within the acceptable range (A260/280: 1.7–2.0). The mean RNA concentration varied across age groups and ethnicities. Among the Igbo participants, the highest mean ± standard error (S.E.) concentration was observed in the 20–25 years age group (1017.12 ± 374.93 ng/µL), while the lowest concentration was recorded within the same ethnicity in the 36–40 years age group (245.20 ± 173.76 ng/µL). For the Yoruba participants, the highest concentration value (825.82 ± 412.82 ng/µL) was observed in the 36–40 years age group, whereas the lowest concentration (341.89 ± 159.29 ng/µL) was recorded in the 31–35 years age group. Purity values across the Yoruba age groups remained within acceptable limits. Table 2 summarizes the descriptive statistics of RNA purity and

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concentration obtained from skin swab samples across age groups and ethnicities.

Pearson correlation of RNA purity and concentration among Igbo, Yoruba and across both ethnic participants

Pearson correlation analysis showed significant relationships between RNA purity and concentration values across selected age groups among the Igbo participants. The concentration and purity values of participants in the 26–30 years and 31–35 years age groups correlated significantly with the concentration values of participants in the 36–40 years age group at the 0.05 level of significance. Additionally, purity values of the 36–40 years age group correlated significantly with concentration values of the 26–30 years age group ($p \leq 0.05$). The Pearson correlation matrix for Igbo participants is presented in Table 3. Among the Yoruba participants, Pearson correlation analysis revealed significant correlations between RNA purity and concentration values across specific age groups. The purity values of the 20–25 years age group correlated significantly with concentration values within the same age group and with those of the 36–40 years age group ($p \leq 0.05$). Furthermore, purity values of the 26–30 years age group correlated significantly with both purity and concentration values of the 20–25 years and 36–40 years age groups ($p \leq 0.05$). The correlation output for the Yoruba participants is shown in Table 4. When data from both ethnic groups were combined, Pearson correlation analysis demonstrated significant associations between selected age groups. The concentration values of Igbo participants in the 31–35 years age group correlated significantly with the concentration values of Yoruba participants in the 20–25 years age group at the $P < 0.05$ significance level. Additionally, purity values of Igbo participants in the 36–40 years age group correlated significantly with purity values of Yoruba participants in the 20–25 years age group at the 0.01 significance level. The correlation matrix across both ethnicities is presented in Table 5.

Quantitative PCR Analysis of miR-130b, miR-138 and miR-155 in Igbo and Yoruba Participants

Quantitative PCR analysis showed detectable expression of miR-130b across all age groups among the Igbo participants, with Cq values consistently within acceptable amplification ranges. The lowest mean ± S.E. Cq value (12.90 ± 6.03) was observed for miR-155 in the 31–35 years age group, while the highest mean ± S.E. Cq value (38.76 ± 0.60) was observed for miR-138 in the 36–40 years age group. Good amplification efficiency ($25 \leq Cq \leq 37$) was observed for miR-130b across all Igbo samples, whereas miR-155 and miR-138 showed greater variability in Cq values across age groups. Descriptive statistics for miRNA expression in Igbo participants are presented in Table 6. Among the Yoruba participants, miR-130b expression was detected across all age groups with consistent Cq values. The lowest mean ± S.E. Cq value (21.57 ± 5.18) was observed for miR-138 in the 20–25 years age group, while the highest mean ± S.E. Cq value (37.81 ± 1.16) was observed for miR-138 in the 26–30 years age group. Similar to the Igbo samples, miR-130b showed stable amplification across all age groups, whereas miR-155 and miR-138 exhibited wider variability. The descriptive analysis for Yoruba participants is shown in Table 7.

Expression Levels of Target microRNAs across Genders and Ethnicities

Figure 1 illustrates the average Cq values of miR-130b, miR-138 and miR-155 across genders and ethnic groups. The expression of miR-130b appeared relatively stable across all genders in both ethnic groups when compared to miR-138 and miR-155, which showed greater variation.

Pearson correlation analysis of miR-130b with GAPDH and ACTB in Igbo participants

Pearson correlation analysis revealed a strong positive correlation between the Cq values of ACTB and miR-130b in the Igbo participants within the 31–35 years age group ($p < 0.01$). However, no significant correlation was observed between the Cq values of miR-130b and GAPDH across all age groups within the Igbo ethnicity ($p > 0.05$).

The correlation matrix for miR-130b, GAPDH and ACTB in Igbo participants is presented in Table 8.

Pearson correlation analysis of miR-155 with GAPDH and ACTB in Igbo participants

A strong positive correlation ($p < 0.01$) was observed between the Cq values of ACTB and miR-155 in the Igbo participants within the 20–25 years and 26–30 years age groups. No statistically significant correlation was observed between miR-155 and GAPDH Cq values across all age groups among the Igbo participants ($p > 0.05$).

The Pearson correlation output for miR-155, GAPDH and ACTB in Igbo participants is shown in Table 9.

3.10 Pearson Correlation Analysis of miR-138 with GAPDH and ACTB in Igbo Participants

Pearson correlation analysis showed a significant positive correlation ($p \leq 0.05$) between the Cq values of ACTB and miR-138 in the Igbo participants within the 26–30 years and 31–35 years age groups. In addition, Cq values of miR-138 between the 26–30 years and 31–35 years age groups correlated significantly ($p \leq 0.05$). No significant correlation was observed between miR-138 and GAPDH across all Igbo age groups ($p > 0.05$).

The correlation results for miR-138, GAPDH and ACTB in Igbo participants are presented in Table 10.

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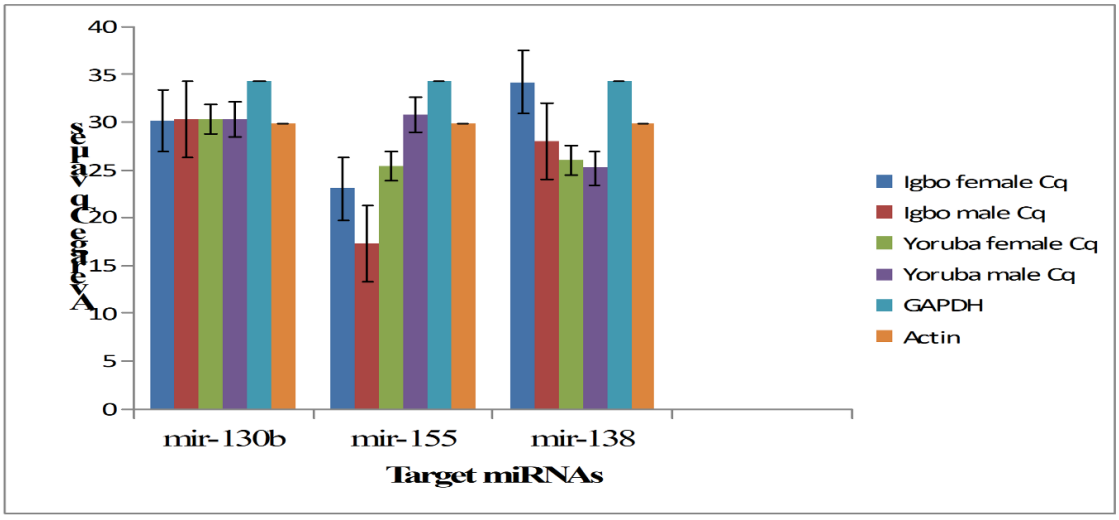

Figure 1. *Average Cq values of miR-130b, miR-138 and miR-155 across genders and ethnicities.*

Table 10: Pearson correlation test output for miR-138 and GAPDH and ACTB Cq values in the Igbo skin swab samples

Parameter	GAPDH	ACTB	Igbo (20-25 years) Cq miR-138	Igbo (26-30 years) Cq miR-138	Igbo (31-35 years) Cq miR-138	Igbo (36-40 years) Cq miR-138
GAPDH	1	0.57	0.28	0.53	-0.58	0.93
		0.62	0.82	0.65	0.60	0.25
ACTB	0.57	1	-0.63	0.10*	-1.00**	0.84
	0.62		0.56	0.03	0.001	0.37
Igbo (20-25 years)	0.28	-0.63	1	0.55	0.62	-0.16
Cq miR-138	0.82	0.53		0.25	0.58	0.84
Igbo (26-30 years)	0.53	0.10*	0.55	1	-0.10*	0.78
Cq miR-138	0.65	0.03	0.25		0.04	0.22
Igbo (31-35 years)	-0.58	-1.00*	0.62	-0.18*	1	-1.85
Cq miR-138	0.60	0.01	0.58	0.04		0.36
Igbo (36-40 years)	0.93	0.84	-0.16	0.78	-0.85	1
Cq miR-138	0.25	0.37	0.84	0.22	0.36	

**Correlation is significant at the 0.05 level . **Correlation is significant at the 0.05 level*

Pearson Correlation Analysis of miR-130b with GAPDH and ACTB in Yoruba Participants

Among the Yoruba participants, Pearson correlation analysis revealed significant correlations between the Cq values of miR-130b in the 26–30 years age group and those of the 31–35 years and 36–40 years age groups ($p \leq 0.05$). However, no significant correlation was observed between miR-130b and the reference genes GAPDH and ACTB across all Yoruba age groups ($p > 0.05$). The Pearson correlation matrix for miR-130b, GAPDH and ACTB in Yoruba participants is shown in Table 11.

Table 11: Pearson correlation test output for miR-130b and GAPDH and ACTB Cq values in the Yoruba skin swab samples

Parameter	GAPDH	ACTB	Yoruba (20-25 years) Cq miR-130b	Yoruba (26-30 years) Cq miR-130b	Yoruba (31-35 years) Cq miR-130b	Yoruba (36-40 years) Cq miR-130b
GAPDH	1	0.57	0.27	-0.95	0.94	0.96
		0.62	0.83	0.21	0.23	0.17
ACTB	0.58	1	0.546	-0.28	0.24	0.33
	0.62		0.21	0.82	0.84	0.79
Yoruba (20-25 years)	0.27	0.95	1	0.05	-0.58	0.001
Cq miR-130b	0.83	0.21		0.97	0.30	1.00

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Yoruba (26-30 years)	-0.95	-0.28	0.05	1	-0.10*	-0.10*
Cq miR-130b	0.21	0.82	0.97		0.02	0.03
Yoruba (31-35 years)	0.94	0.24	-0.58	-0.10*	1	0.10
Cq miR-130b	0.23	0.84	0.30	0.02		0.06
Yoruba (36-40 years)	0.96	0.33	0.001	-0.10*	0.10	1
Cq miR-130b	0.17	0.79	1.00	0.03	0.06	

*. Correlation is significant at the 0.05 level

Pearson Correlation Analysis of miR-155 with GAPDH and ACTB in Yoruba Participants

A significant positive correlation ($p \leq 0.05$) was observed between the Cq values of miR-155 in the Yoruba participants within the 26–30 years and 36–40 years age groups. No statistically significant correlation was detected between miR-155 and the reference genes GAPDH and ACTB across all age groups within the Yoruba ethnicity ($p > 0.05$).

The correlation output for miR-155, GAPDH and ACTB in Yoruba participants is presented in Table 12.

Table 12: Pearson correlation test output for miR-155 and GAPDH and ACTB Cq values in the Yoruba skin swab samples

Parameter	GAPDH	ACTB	Yoruba (20-25 years) Cq miR-155	Yoruba (26-30 years) Cq miR-155	Yoruba (31-35 years) Cq miR-155	Yoruba (36-40 years) Cq miR-155
GAPDH	1	0.57	-0.27	-0.44	-0.98	-0.48
		0.62	0.82	0.71	0.11	0.68
ACTB	0.57	1	-0.95	-0.99	-0.71	-0.99
	0.62		0.21	0.09	0.50	0.07
Yoruba (20-25 years)	-0.28	-0.95	1	0.98	0.52	0.98
Cq miR-155	0.82	0.21		0.12	0.37	0.14
Yoruba (26-30 years)	-0.44	-0.99	0.98	1	0.60	0.10*
Cq miR-155	0.71	0.09	0.12		0.59	0.03
Yoruba (31-35 years)	-0.98	-0.71	0.52	0.60	1	0.63
Cq miR-155	0.11	0.50	0.37	0.59		0.57
Yoruba (36-40 years)	-0.48	-0.99	0.98	0.10*	0.63	1
Cq miR-155	0.68	0.07	0.14	0.03	0.57	

*. *Correlation is significant at the 0.05 level.*

Pearson Correlation Analysis of miR-138 with GAPDH and ACTB in Yoruba Participants

Pearson correlation analysis revealed a significant positive correlation ($p \leq 0.05$) between the Cq values of GAPDH and miR-138 in the Yoruba participants within the 31–35 years age group. No significant correlation was observed between miR-138 and ACTB across all Yoruba age groups ($p > 0.05$). The Pearson correlation matrix for miR-138, GAPDH and ACTB in Yoruba participants is shown in Table 13.

Pearson Correlation Analysis of miR-155 Expression across Genders and Ethnicities

A strong positive correlation ($p < 0.01$) was observed between the expression levels of GAPDH and ACTB across both genders and ethnic groups. No statistically significant correlation was observed between miR-155 expression across genders or between miR-155 and the reference genes ($p > 0.05$). The correlation matrix for miR-155 across genders and ethnicities is shown in Table 15.

Pearson Correlation Analysis of miR-130b Expression across Genders and Ethnicities

Across both genders and ethnic groups, a strong positive correlation ($p < 0.01$) was observed between the expression levels of the reference genes GAPDH and ACTB. A significant correlation was observed between miR-130b expression in Igbo females and Yoruba females ($p < 0.05$). However, no significant correlation was observed between miR-130b expression and the reference genes across genders and ethnicities ($p > 0.05$). The Pearson correlation results for miR-130b across genders and ethnic groups are presented in Table 14

Table 13: Pearson correlation test output for miR-138 and GAPDH and ACTB Cq values in the Yoruba skin swab samples

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Parameter	GAPDH	ACTB	Yoruba (20-25 years) Cq miR-138	Yoruba (26-30 years) Cq miR-138	Yoruba (31-35 years) Cq miR-138	Yoruba (36-40 years) Cq miR-138
GAPDH	1	0.57 0.62	0.48 0.68	-0.95 0.21	-1.00* 0.02	-0.12 0.92
ACTB	0.57 0.62	1	-0.45 0.70	-0.28 0.82	-0.55 0.63	0.75 0.46
Yoruba (20-25 years) Cq miR-138	0.48 0.68	-0.45 0.70	1	-0.73 0.47	0.16 0.80	-0.93 0.24
Yoruba (26-30 years) Cq miR-138	-0.95 0.21	-0.28 0.82	-0.73 0.49	1	0.96 0.19	0.43 0.72
Yoruba (31-35 years) Cq miR-138	-1.00* 0.02	-0.55 0.63	0.16 0.80	0.96 0.19	1	0.15 0.91
Yoruba (36-40 years) Cq miR-138	-0.12 0.92	0.75 0.46	-0.93 0.24	0.43 0.72	0.15 0.91	1

*. *Correlation is significant at the 0.05 level.*

Table 14: Pearson correlation test output for miR-130b and GAPDH and ACTB Cq values in both genders across all ethnicities

Parameter	Igbo female Cq-130b	Igbo male Cq-130b	Yoruba female Cq-130b	Yoruba male Cq-130b	GAPDH	ACTB
Igbo female Cq-130b	1	0.46 0.18	-0.66* 0.04	-0.05 0.89	-0.01 0.98	-0.05 0.90
Igbo male Cq-130b	0.46 0.18	1	-0.08 0.81	0.35 0.27	0.05 0.90	0.01 0.97
Yoruba female Cq-130b	-0.66* 0.04	-0.08 0.81	1	-0.23 0.48	-0.13 0.75	-0.11 0.78
Yoruba male Cq-130b	-0.05 0.89	0.35 0.27	-0.23 0.48	1	-0.38 0.31	-0.37 0.32
GAPDH	-0.01 0.98	0.05 0.90	-0.13 0.75	-0.38 0.31	1	0.10** 0.00
ACTB	-0.048 0.902	0.013 0.97	-0.11 0.78	-0.37 0.32	0.10** 0.00	1

**Correlation is significant at the 0.05 level. **. Correlation is significant at the 0.01 level*

Table 15: Pearson correlation test output for miR-155 and GAPDH and ACTB Cq values in both genders across all ethnicities

Parameter	GAPDH	ACTB	Igbo female Cq-155	Igbo male Cq-155	Yoruba female Cq-155	Yoruba male Cq-155
GAPDH	1	0.10**	0.07	-0.30	0.58	0.12
		0.00	0.86	0.43	0.11	0.75
ACTB	0.10**	1	0.07	-0.30	0.59	0.14
	0.00		0.86	0.43	0.10	0.71

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Igbo female Cq-155	0.07	0.07	1	-0.51	-0.07	0.20
	0.86	0.86		0.14	0.84	0.58
Igbo male Cq-155	-0.30	-0.3	-0.51	1	-0.15	0.14
	0.43	.43	0.14		0.62	0.66
Yoruba female Cq-155	0.58	0.59	-0.07	-0.15	1	-0.04
	0.11	0.10	0.84	0.62		0.89
Yoruba male Cq-155	0.12	0.14	0.20	0.14	-0.04	1
	0.75	0.71	0.58	0.66	0.89	

**. *Correlation is significant at the 0.01 level*

Table 16: Pearson correlation test output for miR-138, GAPDH and ACTB Cq values across genders and ethnicities

Parameter	GAPDH	ACTB	Igbo female Cq-138	Igbo male Cq-138	Yoruba female Cq-138	Yoruba male Cq-138
GAPDH	1	0.10**	-0.18	0.19	0.012	0.490
		0.00	0.65	0.62	0.975	0.181
ACTB	0.10**	1	-0.15	0.18	0.01	0.47
	0.00		0.70	0.65	0.98	0.20
Igbo female Cq-138	-0.18	-0.15	1	-0.09	-0.18	-0.25
	0.65	0.70		0.80	0.61	0.48
Igbo male Cq-138	0.19	0.18	-0.09	1	0.04	0.01
	0.62	0.65	0.80		0.89	0.97
Yoruba female Cq-138	0.01	0.01	-0.18	0.04	1	0.26
	0.98	0.98	0.61	0.89		0.42
Yoruba male Cq-138	0.49	0.47	-0.25	0.01	0.26	1
	0.18	0.20	0.48	0.97	0.42	

**. *Correlation is significant at the 0.01 level*

Pearson Correlation Analysis of miR-138 Expression across Genders and Ethnicities

Pearson correlation analysis showed a strong positive correlation ($p < 0.01$) between the expression levels of GAPDH and ACTB across both genders and ethnic groups. No significant correlation was observed between miR-138 expression across genders or between miR-138 and the reference genes ($p > 0.05$). The Pearson correlation results for miR-138 across genders and ethnicities are presented in Table 16.

Discussion

Skin tissue serves as a critical interface between the human body and the external environment, undergoing continuous cellular renewal throughout life. However, this regenerative capacity declines progressively with age, largely due to the accumulation of senescent cells and molecular damage (Childs et al., 2015; Khalid et al., 2022). Understanding age-related molecular changes in skin is therefore valuable, particularly in forensic investigations where skin cells may be recovered as trace evidence from crime scenes. In the present study, RNA extracted from skin swab samples demonstrated acceptable purity and relatively high concentrations across most samples. These findings indicate that skin swabs are a suitable non-invasive source of RNA for forensic molecular analysis. The consistently high RNA yields observed may be attributed to the efficiency of the TRIzol® extraction protocol, which has been reported to produce higher RNA concentrations compared to column-based extraction kits, particularly when working with low-biomass samples (Trakunram et al., 2019). This supports the applicability of the extraction method used in this study for forensic skin samples. MicroRNAs are increasingly recognised as robust molecular markers due to their short length, resistance to degradation and tissue-specific expression patterns (O'Brien et al., 2018; Iroanya et al., 2022). In this study, miR-130b was detected consistently across all age groups, genders and ethnicities, with relatively stable Cq values. This stability suggests that miR-130b may serve as a reliable endogenous marker for the identification of skin-derived biological material. Previous studies have reported the involvement of miR-130b in keratinocyte function and replicative senescence, supporting its suitability as a skin-specific biomarker (Rivetti di Val Cervo et al., 2012; Gerasymchuk et al., 2020). In contrast, miR-155 and miR-138 exhibited greater variability in expression across age groups and genders. The observed instability of miR-155 expression aligns with earlier reports indicating tissue- and context-specific regulation of this microRNA. Landgraf et al. (2007) reported predominant expression of miR-155 in hematopoietic cells, while reduced expression has been observed

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in non-hematopoietic tissues, including skin. Similarly, Ong et al. (2019) and Storci et al. (2019) reported low or down-regulated expression of miR-155 in ageing tissues, which may explain the variable Cq values observed in this study. The expression pattern of miR-138 also varied across age groups and genders in both ethnicities. Although miR-138 has been reported to be up-regulated in senescent keratinocytes (Rivetti di Val Cervo et al., 2012), it has also been identified as a tumour suppressor microRNA that is down-regulated in several pathological conditions (Shi et al., 2008). The observed influence of environmental conditions on RNA stability in this study is consistent with earlier findings that temperature, substrate, and exposure significantly affect RNA degradation rates in forensic samples (Ibrahim et al., 2021; Sauer et al., 2021; Gauthier et al., 2022). These contrasting biological roles may contribute to its variable expression in skin tissue, particularly across individuals of different ages and sexes. Correlation analyses demonstrated limited interaction between the target miRNAs and the reference genes GAPDH and ACTB, indicating that the expression of miR-130b, miR-155 and miR-138 is largely independent of these housekeeping genes. This observation is consistent with previous findings that miRNA expression does not necessarily correlate with mRNA-based reference genes due to differences in regulatory mechanisms (Mancini et al., 2014). The strong correlation observed between GAPDH and ACTB across samples confirms their suitability as reference genes in this study. Importantly, linear regression analysis revealed a significant association between miR-130b expression and age in Yoruba females, suggesting a potential gender- and ethnicity-specific regulatory pattern. Additionally, miR-155 and miR-138 showed significant associations with age across all genders, indicating their potential utility as age-predictive biomarkers. These findings support the growing body of evidence that miRNA expression is influenced by both biological sex and age, likely reflecting hormonal regulation and sex-specific gene expression patterns (Dai & Ahmed, 2014; Lin et al., 2022). From a forensic perspective, the ability to estimate age and gender from skin swab samples could provide valuable investigative intelligence, particularly in cases involving touch DNA or trace biological material. The population-specific focus of this study is especially relevant, as genetic diversity and environmental factors can influence molecular ageing patterns (Ludwig et al., 2016). The findings therefore contribute important baseline data for forensic age estimation research within Nigerian populations.

Conclusion

This study evaluated the stability and expression patterns of selected skin-specific microRNAs (miR-130b, miR-138, and miR-155) for age and gender prediction among individuals from Igbo and Yoruba ethnic groups in Nigeria. The findings demonstrate that skin swab samples yield RNA of sufficient quality and concentration for downstream molecular analysis, confirming their suitability as non-invasive forensic samples. Among the microRNAs assessed, miR-130b showed consistent and stable expression across age groups, genders, and ethnicities, highlighting its potential usefulness as a reliable marker for identifying skin-derived biological material. In contrast, miR-155 and miR-138 exhibited variable expression patterns with notable associations to age and gender, suggesting their possible application in forensic age and gender estimation. The observed expression trends underscore the influence of biological and population-specific factors on microRNA regulation and emphasize the importance of developing population-relevant forensic biomarkers, particularly for underrepresented populations. Although the study provides valuable insights into the forensic applicability of skin microRNAs, limitations related to sample size were noted. Future studies involving larger and more diverse populations, broader age ranges, and expanded panels of skin-specific microRNAs are recommended to further enhance the reliability and forensic utility of these biomarkers.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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Result Tables

Table 2: Descriptive statistics of RNA purity and concentration in skin swab samples.

Parameter	Range	Minimum	Maximum	Mean ± S.E.	Variance
Igbo (20-25 years) Purity	0.48	1.62	2.10	1.83 ± 0.05	0.03

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Igbo (20-25 years)	Conc. (ng/µL)	3542.77	18.90	3561.67	1017.12 ± 374.93	1686853.03
Igbo (26-30 years)	Purity	0.12	1.96	2.08	2.03 ± 0.02	0.002
Igbo (26-30 years)	Conc. (ng/µL)	1871.86	337.87	2209.73	705.92 ± 301.33	544825.43
Igbo (31-35 years)	Purity	0.25	1.68	1.93	1.78 ± 0.08	0.02
Igbo (31-35 years)	Conc. (ng/µL)	714.30	65.60	779.90	394.88 ± 208.08	129886.89
Igbo (36-40 years)	Purity	0.12	1.64	1.76	1.71 ± 0.02	0.002
Igbo (36-40 years)	Conc. (ng/µL)	738.20	17.50	755.70	245.20 ± 173.76	120773.74
Yoruba (20-25 years)	Purity	0.50	1.50	2.00	1.73 ± 0.04	0.02
Yoruba (20-25 years)	Conc. (ng/µL)	2137.44	2.73	2140.17	447.76 ± 196.92	542883.1
Yoruba (26-30 years)	Purity	0.38	1.74	2.12	1.96 ± 0.11	0.04
Yoruba (26-30 years)	Conc. (ng/µL)	464.17	378.63	842.80	574.10 ± 138.91	57885.42
Yoruba (31-35 years)	Purity	0.82	1.73	2.55	2.02 ± 0.15	0.11
Yoruba (31-35 years)	Conc. (ng/µL)	841.67	1.90	843.57	341.89 ± 159.29	126871.13
Yoruba (36-40 years)	Purity	0.16	1.68	1.84	1.77 ± 0.05	0.007
Yoruba (36-40 years)	Conc. (ng/µL)	1287.30	2.37	1289.67	825.82 ± 412.82	511273.04

Table 3: Pearson correlation analysis output of the purity and concentration values of skin RNA extracts among the Igbo participants

Parameter	Igbo (20-25 years) Purity	Igbo (20-25 years) Conc. (ng/µL)	Igbo (26-30 years) Purity	Igbo (26-30 years) Conc. (ng/µL)	Igbo (31-35 years) Purity	Igbo (31-35 years) Conc. (ng/µL)	Igbo (36-40 years) Purity	Igbo (36-40 years) Conc. (ng/µL)
Igbo (20-25 years) Purity	1	-0.04 0.90	0.76 0.08	-0.49 0.32	-0.98 0.12	0.10 0.94	0.51 0.50	-0.64 0.36
Igbo (20-25 years) Conc. (ng/µL)	-0.04 0.90	1	0.05 0.93	-0.37 0.47	-0.99 0.10	0.07 0.96	0.49 0.51	-0.64 0.36
Igbo (26-30 years) Purity	0.76 0.08	0.05 0.93	1	-0.29 0.58	-0.97 0.15	0.13 0.91	0.75 0.25	-0.32 0.68
Igbo (26-30 years) Conc. (ng/µL)	-0.49 0.32	-0.37 0.47	-0.28 0.58	1	0.97 0.15	-0.13 0.92	0.22 0.78	0.98* 0.02
Igbo (31-35 years) Purity	-0.98 0.12	-0.99 0.10	-0.97 0.15	0.97 0.15	1	0.10 0.94	-0.10 0.94	1.00* 0.02
Igbo (31-35 years) Conc. (ng/µL)	0.10 0.94	0.07 0.96	0.13 0.91	-0.13 0.92	0.10 0.94	1	0.98 0.13	0.07 0.96

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Igbo (36-40 years) Purity	0.51 0.50	0.49 0.51	0.75 0.25	0.22 0.78	-0.10 0.94	0.98 0.13	1	0.31 0.69
Igbo (36-40) Conc. (ng/µL)	-0.64 0.36	-0.64 0.36	-0.32 0.68	0.98* 0.02	1.00* 0.02	0.07 0.96	0.314 0.686	1

*. *Correlation is significant at the 0.05 level.*

Table 4: Pearson correlation analysis output of the purity and concentration values of skin RNA extracts among the Yoruba participants

Parameter	Yoruba (20-25 years) Purity	Yoruba (20-25 years) Conc. . (ng/µL)	Yoruba (26-30 years) Purity	Yoruba (26-30 years) Conc. (ng/µL)	Yoruba (31-35 years) Purity	Yoruba (31-35 years) Conc. (ng/µL)	Yoruba (36-40 years) Purity	Yoruba (36-40 years) Conc. (ng/µL)
Yoruba (20-25 years) Purity	1	0.61* 0.02	-0.96 0.18	0.97 0.16	-0.51 0.38	0.002 0.10	-0.95 0.10	-0.10* 0.05
Yoruba (20-25 years) Conc. (ng/µL)	0.61* 0.02	1	0.10* 0.03	-0.83 0.38	-0.70 0.19	0.43 0.48	1.00* 0.02	0.967 0.17
Yoruba (26-30 years) Purity	-0.96 0.18	0.10* 0.03	1	-0.86 0.35	-0.92 0.26	0.40 0.74	1.00* 0.02	0.98 0.14
Yoruba (26-30 years) Conc. (ng/µL)	0.97 0.16	-0.83 0.38	-0.86 0.35	1	0.99 0.09	0.13 0.92	-0.84 0.36	-0.95 0.21
Yoruba (31-35 years) Purity	-0.51 0.38	-0.68 0.19	-0.92 0.26	0.99 0.09	1	-0.44 0.43	-0.91 0.27	-0.98 0.12
Yoruba (31-35 years) Conc. (ng/µL)	0.002 0.10	0.43 0.48	0.40 0.74	0.13 0.92	-0.44 0.46	1	0.42 0.72	0.20 0.87
Yoruba (36-40 years) Purity	-0.95 0.20	1.00* 0.02	1.00* 0.02	-0.84 0.36	-0.91 0.27	0.42 0.72	1	0.97 0.15
Yoruba (36-40 years) Conc. (ng/µL)	-0.10* 0.05	0.97 0.17	0.98 0.14	-0.95 0.21	-0.98 0.12	0.20 0.87	0.97 0.15	1

*. *Correlation is significant at the 0.05 level*

Table 5: Pearson correlation analysis output of the purity and concentration values of skin RNA extracts across all ethnicities

Parameter	Yoruba (20-25 years) Purity	Yoruba (20-25 years) Conc. (ng/µL)	Yoruba (26-30 years) Purity	Yoruba (26-30 years) Conc. (ng/µL)	Yoruba (31-35 years) Purity	Yoruba (31-35 years) Conc. (ng/µL)	Yoruba (36-40 years) Purity	Yoruba (36-40 years) Conc. (ng/µL)
Igbo (20-25 years) Purity	0.13 0.69	0.25 0.43	-0.20 0.87	0.68 0.53	-0.29 0.64	0.65 0.23	-0.18 0.89	-0.40 0.74
Igbo (20-25 years) Conc. (ng/µL)	0.25 0.43	-0.16 0.63	-0.17 0.89	0.65 0.55	-0.29 0.64	0.66 0.22	-0.14 0.91	-0.37 0.76
Igbo (26-30 years) Purity	0.40 0.43	0.25 0.64	-0.24 0.85	0.70 0.50	-0.57 0.32	0.83 0.08	-0.21 0.86	-0.44 0.71
Igbo (26-30 years) Conc. (ng/µL)	0.34 0.51	0.44 0.39	0.24 0.85	-0.70 0.50	-0.53 0.36	-0.48 0.41	0.21 0.87	0.44 0.71
Igbo (31-35 years) Purity	-0.29 0.81	-0.04 0.97	0.01 0.10	-0.52 0.65	-0.40 0.74	-0.91 0.27	-0.02 0.99	0.22 0.86

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Igbo (31-35 years) Conc. (ng/µL)	0.93 0.25	-0.10* 0.04	-0.10 0.07	0.80 0.41	0.88 0.32	-0.49 0.67	-0.10 0.05	-0.95 0.20
Igbo (36-40 years) Purity	0.10** 0.004	0.11 0.90	-0.10 0.06	0.90 0.29	-0.75 0.25	0.33 0.67	-0.99 0.08	-0.99 0.08
Igbo (36-40 years) Conc. (ng/µL)	0.26 0.74	0.20 0.80	0.04 0.98	-0.55 0.63	-0.50 0.50	-0.51 0.49	0.01 0.99	0.25 0.84

*. *Correlation is significant at the 0.05 level* **. *Correlation is significant at the 0.01 level*

Table 6: Descriptive analysis output for miR-130b, miR-138 and miR-155 in Skin Swab Samples of the Igbo Participants.

rameter	Range	Minimum	Maximum	Mean ± S.E.
Igbo (20-25 years) Cq MIR-130B	1.49	29.70	31.19	30.18 ± 0.11
Igbo (26-30 years) Cq MIR-130B	0.77	30.01	30.78	30.20 ± 0.12
Igbo (31-35 years) Cq MIR-130B	0.54	30.37	30.91	30.56 ± 0.17
Igbo (36-40 years) Cq MIR-130B	1.10	29.51	30.61	30.02 ± 0.23
Igbo (20-25 years) Cq MIR-155	34.95	1.59	36.54	22.08 ± 3.05
Igbo (26-30 years) Cq MIR-155	31.87	4.59	36.46	17.08 ± 6.03
Igbo (31-35 years) Cq MIR-155	20.54	3.71	24.25	12.90 ± 6.03
Igbo (36-40 years) Cq MIR-155	29.25	9.00	38.25	23.25 ± 6.05
Igbo (20-25 years) Cq MIR-138	39.41	0.00	39.41	28.09 ± 4.90
Igbo (26-30 years) Cq MIR-138	37.41	3.13	40.54	32.17 ± 5.85
Igbo (31-35 years) Cq MIR-138	40.19	0.00	40.19	25.70 ± 1.289
Igbo (36-40 years) Cq MIR-138	2.83	37.41	40.24	38.76 ± 0.60
GAPDH	5.49	30.70	36.19	34.30 ±1.80
ACTB	21.63	16.02	37.65	29.76 ± 6.90

Table 7: Descriptive analysis output for miR-130b, miR-138 and miR-155 in Skin Swab Samples of the Yoruba Participants.

ameters	Range	Minimum	Maximum	Mean ± S.E.
Yoruba (20-25 years) Cq MIR-130B	1.15	29.75	30.90	30.35 ± 0.08
Yoruba (26-30 years) Cq MIR-130B	0.53	30.02	30.55	30.25 ± 0.16
Yoruba (31-35 years) Cq MIR-130B	0.81	30.23	31.04	30.50 ± 0.14
Yoruba (36-40 years) Cq MIR-130B	1.62	28.64	30.26	29.58 ± 0.49
Yoruba (20-25 years) Cq MIR-155	37.15	1.96	39.11	26.30 ± 3.12
Yoruba (26-30 years) Cq MIR-155	26.87	13.04	39.91	30.06 ± 8.55
Yoruba (31-35 years) Cq MIR-155	18.45	20.13	38.58	31.06 ± 3.73
Yoruba (36-40 years) Cq MIR-155	28.27	10.09	38.36	28.39 ± 9.16
Yoruba (20-25 years) Cq MIR-138	38.99	0.00	38.99	21.57 ± 5.18
Yoruba (26-30 years) Cq MIR-138	3.92	36.11	40.03	37.81 ± 1.16
Yoruba (31-35 years) Cq MIR-138	38.47	0.00	38.47	22.78 ± 9.30
Yoruba (36-40 years) Cq MIR-138	0.51	36.85	37.36	37.13 ± 0.15
GAPDH	5.49	30.70	36.19	34.30 ± 1.80
ACTB	21.63	16.02	37.65	29.70 ± 6.90

Table 8: Pearson correlation test output for miR-130b and GAPDH and ACTB Cq values in the Igbo skin swab samples

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Parameter	Igbo (20-25 years) Cq miR-130b	Igbo (26-30 years) Cq miR-130b	Igbo (31-35 years) Cq miR-130b	Igbo (36-40 years) Cq miR-130b	GAPDH	ACTB
Igbo (20-25 years) Cq miR-130b	1	0.66 0.15	0.14 0.91	-0.61 0.39	-0.73 0.48	0.15 0.90
Igbo (26-30 years) Cq miR-130b	0.66 0.15	1	-0.236 0.85	-0.48 0.52	-0.93 0.24	-0.22 0.86
Igbo (31-35 years) Cq miR-130b	0.14 0.91	-0.24 0.85	1	-0.73 0.48	0.58 0.61	1.00** 0.01
Igbo (36-40 years) Cq miR-130b	-0.61 0.39	-0.48 0.52	-0.73 0.48	1	0.13 .092	-0.74 0.47
GAPDH	-0.73 0.48	-0.93 0.24	0.58 0.61	0.13 0.92	1	0.57 0.62
ACTB	0.15 0.90	-0.22 0.86	1.00** .008	-0.74 0.47	0.57 0.62	1

***. Correlation is significant at the 0.01 level.*

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Table 9: Pearson correlation test output for miR-155 and GAPDH and ACTB Cq values in the Igbo skin swab samples

Parameter	GAPDH	ACTB	Igbo (20-25 years) Cq miR-155	Igbo (26-30 years) Cq miR-155	Igbo (31-35 years) Cq miR-155	Igbo (36-40 years) Cq miR-155
GAPDH	1	0.57 0.62	-0.56 0.62	-0.57 0.61	0.21 0.87	-0.96 0.18
ACTB	0.57 0.62	1	-1.00** 0.01	-1.000** 0.001	0.92 0.25	-0.31 0.80
Igbo (20-25 years) Cq miR-155	-0.56 0.62	-1.00** 0.008	1	0.66 0.16	-0.93 0.25	0.13 0.87
Igbo (26-30 years) Cq miR-155	-0.57 0.61	-1.00** 0.001	0.66 0.16	1	-0.92 .254	0.59 0.41
Igbo (31-35 years) Cq miR-155	0.21 0.87	0.92 0.25	-0.93 0.25	-0.92 0.25	1	0.08 0.95
Igbo (36-40 years) Cq miR-155	-0.96 0.18	-0.31 0.78	0.13 0.87	0.59 0.41	0.08 0.95	1

***. Correlation is significant at the 0.01 level.*

Table 17: Linear regression model for the association of miR-130b within all participants

Variable	B	S. E.	Beta	T	*P*	Model
(Constant)	154.85	151.88	-0.26	1.02	0.34	Y = 154.85 + (-4.08*x)
IF (MiR-130b)	-4.08	5.04		-0.81		
(Constant)	43.88	126.71	-0.04	0.35	0.74	Y = 43.88 + (-0.62*x)
IM (MiR-130b)	-0.62	4.19		-0.15		
(Constant)	206.20	83.75	-0.54	2.46	0.03	Y = 206.20 + (-5.87*x)
YF (MiR-130b)	-5.87	2.77		-2.12		
(Constant)	18.94	218.22	0.01	0.09	0.93	Y = 18.94 + (0.21*x)
YM (MiR-130b)	0.21	7.21		0.03		

Key: IF=Igbo female, IM=Igbo man, YF=Yoruba female, YF= Yoruba man, y= gender, x= Ct value of MiR-130b, β =Correlation coefficient, SE=Standard Error

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Table 18: Linear regression model for the association of miR-155 within all participants

Variable	B	S. E.	Beta	t	*P*	Model
(Constant)	21.51	2.09	0.89	10.29	*0.00*	Y = 21.51 + (0.46*m)
miR-155b (IF)	0.46	0.08		5.58		
(Constant)	31.42	1.481	-0.82	21.22	*0.00*	Y = 31.42 + (-0.36*m)
miR-155b (IM)	-0.36	0.071		-5.09		
(Constant)	27.75	4.47	0.06	6.21	*0.00*	Y = 27.75 + (0.03*m)
miR-155b (YF)	0.03	0.16		0.21		
(Constant)	18.04	5.21	0.42	3.46	*0.01*	Y = 18.04 + (0.23*m)
miR-155b (YM)	0.23	0.16		1.46		

Key: IF=Igbo female, IM=Igbo man, YF=Yoruba female, YF= Yoruba man, y= gender, m= Ct value of miR-155b, β =Correlation coefficient, SE=Standard Error

Table 19: Linear regression model for the association of miR-138 within all participants

Variable	B	S. E.	Beta	t	*P*	Model
(Constant)	30.71	6.29		4.88		Y = 30.71 + (0.04*n)
miR138b (IF)	0.04	0.17	0.08	0.22	0.001	
(Constant)	24.60	2.85		8.63		Y = 24.60 + (0.02*n)
miR138b (IM)	0.02	0.09	0.07	0.24	0.00	
(Constant)	27.76	3.29		8.44		Y = 27.76 + (0.03*n)
miR138b (YF)	0.03	0.11	0.09	0.31	0.00	