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DNA Methylation Profiling: Unveiling Population-Specific and Gender-Based Variations for Forensic Applications

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Abstract:
Background: The forensic science landscape has been significantly transformed by the advent of DNA methylation analysis, offering unparalleled precision in body fluid identification, age estimation, and distinguishing between monozygotic twins. This review delves into the historical evolution, methodological advancements, and the pivotal role of DNA methylation in forensic applications, underscoring its potential in addressing complex forensic challenges.
Results: The study detected significant differences in the expression of *LINE-1* and *FGF7* in blood, and *MT2A* and *FGF7* in saliva, between Egyptian and Yemeni populations, P-value< 0.05. Profound differences were observed in the expression of *MT2A*, *LINE-1*, and *FGF7* in both blood and saliva samples between males and females, P-value< 0.05. The markers examined in this study demonstrated good sensitivity and specificity for identifying the origin of blood and saliva samples based on nationality and gender, underscoring their potential forensic and biomedical applications.
Conclusion: this study offers appreciated visions into the influence of population-specific and gender-based factors on DNA methylation profiles, contributing to the growing body of evidence supporting the use of these molecular markers in forensic investigations and personalized medicine. The findings emphasize the importance of considering these variables in the interpretation and application of DNA methylation data for effective and tailored diagnostic and therapeutic strategies.

Keywords: DNA methylation, Forensic epigenetics, Body fluid identification, Biomarkers, Forensic science.

1. Introduction

Epigenetics, a fascinating and rapidly evolving field of genetics, primarily concerns understanding the various mechanisms that regulate gene expression without altering the underlying DNA sequence. DNA methylation is one of this realm's most critical and extensively studied mechanisms. This process involves adding a methyl group to the DNA, specifically to the cytosine or adenine nucleotides, a modification that plays a vital role in controlling gene expression (Jones & Takai, 2001).

DNA methylation is a key epigenetic modification that profoundly impacts various biological processes and states. It is often associated with the repression of gene transcription when occurring in the promoter regions of genes. The addition of methyl groups typically happens at the 5-carbon position of the cytosine ring within the CpG dinucleotides, which are regions where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence of bases along its 5' → 3' direction (Smith & Meissner, 2013). These methyl groups can inhibit gene expression by hindering the binding of transcription factors to the DNA or recruiting proteins associated with gene repression (Bird's 2002).

In addition, The discovery of DNA methylation as a stable epigenetic marker has opened new avenues for forensic analysis, providing insights into the source of biological samples and their conditions (Jobling and Gill, 2004). Forensic science has continually evolved, integrating molecular biology techniques to improve the accuracy and reliability of crime scene investigations. DNA methylation has emerged as a critical biomarker in forensic science. Its stability and tissue-specific patterns facilitate the identification of bodily fluids, estimating biological age, and differentiating individuals, including monozygotic twins (Staunstrup et al., 2017).

The ability to accurately identify the type of body fluid at a crime scene can significantly impact the interpretation of forensic evidence. DNA methylation patterns offer a high level of specificity in distinguishing between blood, saliva, semen, and other bodily fluids, contributing to the reconstruction of crime scenes and the establishment of the events leading to the crime. In addition, age estimation through DNA methylation analysis has shown promise in forensic medicine, providing a molecular approach to determining the age of unidentified remains. Additionally, DNA methylation variability can assist in differentiating between individuals, particularly in challenging cases involving monozygotic twins (Bulter, 2009; Anđelinović et al., 2005).

Recent technological advances have enhanced the sensitivity and specificity of DNA methylation analysis. Techniques such as bisulfite sequencing and methylation-sensitive high-resolution melting (MS-HRM) allow for the precise quantification of methylation levels, enabling the development of forensic applications (Vidaki et al., 2013; Lin et al., 2016). In the revolutionary era of forensic DNA analysis, Restriction Fragment Length Polymorphism (RFLP) was the foremost technique utilized to dissect the complexities of genetic material retrieved from crime scenes. This method entailed the cleavage of double-stranded DNA with restriction endonucleases, followed by the segregation of resultant fragments via electrophoresis.

Notably, specific genomic DNA fragments were identified using either radioactive or chemiluminescent probes, binding distinctively to sequences of interest (Jeffreys et al., 1985). However, RFLP's dependency on substantial quantities of intact DNA and its protracted, labor-intensive process rendered it obsolete with the advent of more refined, time-efficient technologies. This transition underscores the dynamic evolution of forensic methodologies, pivoting towards techniques that enhance sensitivity, reduce operational times, and accommodate the analysis of degraded samples, thereby expanding the horizon of genetic evidence's utility in criminal investigations (Butler, 2009).

Additionally, accurately detecting body fluids in situ non-destructively is essential to protect the sample and preserve the DNA evidence. The development of a comprehensive body fluid identification system capable of performing multiplex reactions on minimal sample sizes, represents a significant leap forward in forensic science, offering unparalleled specificity and sensitivity in evidence analysis (Vidaki et al., 2013). Researchers proposed that DNA methylation might solve the forensic mystery of differentiating monozygotic (MZ) twins because MZ twins display varying degrees of phenotypic differences (Bell et al., 2011; Li et al., 2013). Age estimation is also an essential issue in forensic investigation, and forensic scientists have resorted to analyzing DNA methylation patterns to estimate the age involved in criminal investigations or the possible biological age of tissues at the time of death (Lynnerup et al., 2010).

Despite its potential, the application of DNA methylation in forensic science faces challenges, including the need for comprehensive databases of methylation markers for various tissues and conditions. Future research should focus on expanding these databases and improving the efficiency and cost-effectiveness of methylation analysis techniques.

Therefore, this study focuses on utilizing epigenetic markers (*LINE-1*, *MGMT*, *MT2A*, and *FGF7*) to precisely identify biological fluids, specifically blood and saliva, commonly encountered at crime scenes. The selection of these markers is predicated on their established relevance in epigenetic studies, offering a robust framework for forensic analysis.

2. Participants and Methods

2.1. Participants

The participant selection process for this study adhered to well-defined criteria to ensure the integrity and relevance of the research findings. The inclusion criteria specified that participants should be normal, healthy individuals with blood and saliva samples exhibiting no signs of disease. This criterion was crucial in maintaining the homogeneity of the study group. The quality of saliva was assessed to be normal and disease-free for both sexes.

Conversely, individuals were excluded from the study if they had any form of blood disease or coagulopathy or were diagnosed with malignant tumors to prevent confounding variables that could skew the results. Furthermore, all participants who met the inclusion criteria and were selected for the study provided written consent. This consent was obtained after they were fully informed about the study's objectives and procedures, ensuring ethical compliance and participant awareness. The inclusion and exclusion criteria, along with the consent process, were critical components in assembling a representative and ethically sound participant group for the study.

2.2. Study Design

96 samples were collected, comprising equal numbers of blood and saliva samples, each containing 48 specimens. The collection of these samples was conducted with careful consideration of the age, sex, and country of origin of the volunteers, factors crucial for the diversity and representativeness of the study.

The samples were further categorized based on gender and country. This categorization resulted in two primary groups, each consisting of 48 samples: one group for male and another for female participants. Within these groups, the samples were equally divided based on the type of specimen – blood and saliva.

Consequently, there were 24 blood samples from females and 24 from males for each country involved in the study. This division pattern was replicated for saliva samples, ensuring an equal and parallel distribution of blood and saliva specimens across genders. This systematic classification of samples was pivotal for facilitating a gender-balanced and geographically diverse analysis, essential for the robustness and relevance of the study's outcomes.

2.3.Collection of Samples

Blood samples were collected from 100 people from different places - the University Hospitals in Banha, Cairo, and Tanta, as well as 24 samples from Iraqi volunteers residing in Egypt and some from Egyptian residents of Yemeni and Sudanese nationalities. All samples were from males and females ranging from 20 to 60 years old. Samples were collected over 6 months to identify similarities and differences through blood and saliva samples and determine gender, age, and smoking effects. The samples were divided into four groups: Group A consisted of 24 blood and saliva samples from 3 clans in Egypt. Group B: 24 blood and saliva samples from Iraq from 3 different clans. Group C: 24 blood and saliva samples from Sudan from 3 clans. Group D: 24 blood and saliva samples from Yemen from 3 clans.

2.4.DNA Extraction

DNA was extracted from whole blood using the Qia-amplification DNA extraction kit (Qiagen, USA). The principle is that DNA binds to the Qia-amp silica-gel membrane while contaminants pass through. PCR inhibitors like divalent cations and proteins are removed in wash steps, leaving pure DNA to be eluted. DNA concentration was measured spectrophotometrically and calculated based on the OD260 reading. In addition, Bisulfite treatment was done to convert unmethylated cytosines to uracils while leaving methylated cytosines unchanged. This process allows the differentiation of methylated and unmethylated sequences.

2.5. Quantitative Real-Time PCR

Bisulfite-modified DNA was used as a template for qPCR using the QuantiTect SYBR Green PCR kit on a Rotor-Gene thermocycler. Primers were designed for the genes of interest (*MGMT, MT2A, LINE-1, FGF7*) and the β-actin reference gene. Table 1 shows the Primer sequences of the genes examined in the study.

Table 1. Primers used for the analysis of DNA methylation in all studied samples

Genes	Primer sequence (5' ® 3')	accession number
MGMT	**F**: CGAATATACTAAAACAACCCGCG	NG027726.1
	R: AATCCTCGCGATACGCACCGTTTACG	
MT2A	**F**: AATTTGGCGAAGCGTTAAGGCGCGCGA	AB012922.1
	R: AAAACAAAACCCACTACTCAACTCAAATC	
LINE-1	**F**: TTGAGTTGTGGTGGGTTTTATTTAG	X58075.1
	R: TCATCTCACTAAAAAATACCAAACA	
FGF7	**F**:GGGTTTATATGTATTTTTGGTGGT	AC087742.9
	R: CATTATATACTCCTCAAAACACACAC	
B-actin	**F**: TGGTGATGGAGGAGGTTTAGTAAGT	AP013035.1
	R:ACCACCACCCAACACACAATAACAAACACA	

2.6. Methylation Quantitation

Methylation levels were quantified using the ΔΔCt method relative to a universally methylated DNA standard and normalized to the β-actin reference gene:

$\Delta\Delta Ct$ = [(Ct target - Ct reference)sample - (Ct target - Ct reference)control]

Relative quantitation (RQ) = $2^{-\Delta\Delta Ct}$. This allowed accurate quantitative methylation assessment of the genes of interest in samples versus the control.

2.7. Data analysis

Data analysis was conducted using IBM SPSS software package version 27.0 (Armonk, NY: IBM Corp). Quantitative data were presented through mean and standard deviation. The comparison of two quantitative variables employed the independent sample t-test, while the comparison of more than two quantitative variables was assessed through the one-way ANOVA test. The post-hock test was conducted via the Benferroni test. Additionally, the ROC curve analysis was conducted to determine the diagnostic accuracy of the biomarkers. Statistical significance was assigned to values below 0.05.

3. Results

3.1. Epigenetic Marker expression

Table 1 shows the comparison between the four markers in the blood and saliva regarding nationality. First, for blood, statistically significant differences were observed in the expression of *LINE-1* and FGF7, P-value= 0.023 and 0.025, respectively. *LINE-1* was overexpressed in the Egyptian (0.335 ± 0.045) than in Yeminian (0.190 ± 0.046), and *FGF7* was also overexpressed in the Egyptian (0.475 ± 0.168) than in Yeminian (0.108 ± 0.024), P-values< 0.05. Second, for saliva, statistically significant differences were observed in the expression of *MT2A* and *FGF7*, P-value= 0.013 and 0.002, respectively. *MT2A* was overexpressed in the Yeminian (4.06 ± 0.152) than in the Egyptian (3.35 ± 0.281), while *FGF7* was overexpressed in the Egyptian (0.285 ± 0.021) and the Sudanian (0.312 ± 0.134) than in Yeminian (0.142 ± 0.087), P-values< 0.05.

Table 2. Comparison between the four markers in the blood and saliva regarding the nationality

Epigenetic Marker	Egyptian	Yeminian	Sudanian	Iraqian	P-value
Blood					
MGMT	1.45 ± 0.328	1.47 ± 0.207	1.69 ± 0.277	1.77 ± 0.242	0.072
MT2A	1.66 ± 0.110	1.43 ± 0.059	1.55 ± 0.176	1.55 ± 0.188	0.153
LINE-1	0.335 ± 0.045#	0.190 ± 0.046#	0.266 ± 0.084	0.275 ± 0.095	0.023*
FGF7	0.475 ± 0.168#	0.108 ± 0.024#	0.270 ± 0.245	0.316 ± 0.214	0.025*
Saliva					
MGMT	0.263 ± 0.068	0.262 ± 0.037	0.269 ± 0.097	0.227 ± 0.133	0.745
MT2A	3.35 ± 0.281#	4.06 ± 0.152#	3.84 ± 0.334	3.81 ± 0.465	0.013*
LINE-1	1.81 ± 0.099	1.52 ± 0.107	1.70 ± 0.232	1.65 ± 0.283	0.137
FGF7	0.285 ± 0.021#	0.142 ± 0.087#	0.312 ± 0.134#	0.203 ± 0.052	0.002*

Values represent Mean ± SD. The P-value is calculated by a one-way ANOVA test. * Significant at <0.05. # Significance of the post-Hock test comparison.

On the other hand, Table 3 shows the comparison between the four markers in the blood and saliva regarding gender. For blood, statistically significant differences were observed in the expression

of *MT2A, LINE-1*, and *FGF7*, P-value< 0.001. *MT2A* was overexpressed in the females (1.68 ± 0.089) than in the males (1.39 ± 0.068), *LINE-1* was also overexpressed in the females (0.337 ± 0.047) than in the males (0.189 ± 0.037), and *FGF7* was also overexpressed in the females (0.451 ± 0.193) than in the males (0.114 ± 0.020), P-values< 0.05. similarly, statistically significant differences were observed in the expression of *MT2A, LINE-1*, and *FGF7* in saliva, P-value< 0.001. *MT2A* was overexpressed in the males (4.07 ± 0.159) than in the females (3.52 ± 0.383). In contrast, *LINE-1* was overexpressed in the females (1.84 ± 0.131) than in the males (1.47 ± 0.107), and *FGF7* was also overexpressed in the females (0.302 ± 0.103) than in the males (0.174 ± 0.058), P-values< 0.05.

Table 3. Comparison between the four markers in the blood regarding gender

Epigenetic Marker	Female	Male	P-value
Blood			
MGMT	1.62 ± 0.283	1.66 ± 0.303	0.721
MT2A	1.68 ± 0.089	1.39 ± 0.068	<0.001*
LINE-1	0.337 ± 0.047	0.189 ± 0.037	<0.001*
FGF7	0.451 ± 0.193	0.114 ± 0.020	<0.001*
Saliva			
MGMT	0.264 ± 0.098	0.240 ± 0.098	0.604
MT2A	3.52 ± 0.383	4.07 ± 0.159	<0.001*
LINE-1	1.84 ± 0.131	1.47 ± 0.107	<0.001*
FGF7	0.302 ± 0.103	0.174 ± 0.058	<0.001*

Values represent Mean ± SD. The P-value is calculated by an independent sample t-test. * Significant at <0.05.

3.2.Accuracy of epigenetic markers regarding nationality

As shown in Table 4 and Figure 1, selected markers showed good sensitivity and specificity for blood and saliva identification regarding nationality. Results suggested that *LINE-1* and *FGF7* are good molecular markers for blood identification regarding nationality. *FGF7* is a good molecular marker for saliva identification regarding nationality.

Table 4. Specificity and sensitivity of significant epigenetic markers regarding nationality in blood and saliva identification.

Epigenetic markers	AUC	P-value	95% Confidence Interval		Cut-off value	Sensitivity (%)	Specificity (%)
			Lower Bound	Upper bound			
LINE-1 (blood)	0.798	0.025*	0.645	0.956	0.325	83.3	80.8
FGF7 (blood)	0.814	0.018*	0.664	0.964	0.230	100	69.2

MT2A (saliva)	0.737	0.074	0.570	0.905	3.95	83.3	65.4
FGF7 (saliva)	0.734	0.036*	0.528	0.941	0.275	60	72.7

AUC is the area under the curve. * Significant at <0.05.

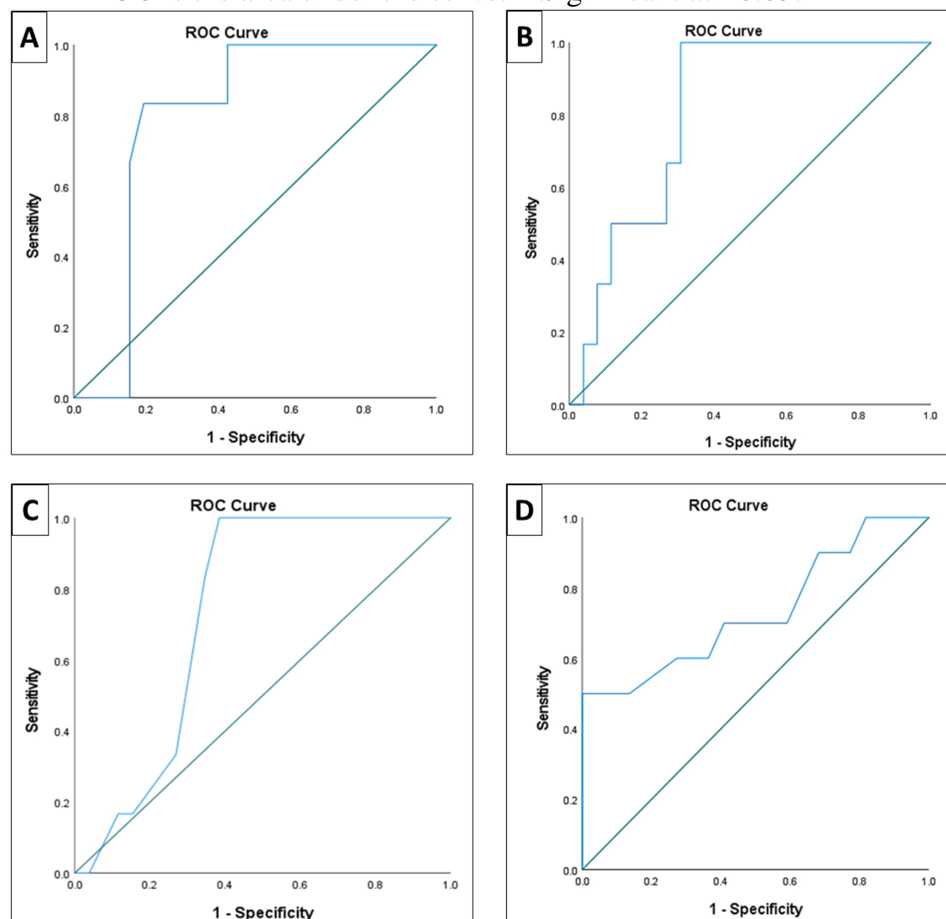

Figure 1. ROC curve for nationality identification using (A) *LINE-1* (blood). (B) *FGF7* (blood). (C) *MT2A* (saliva). (D) *FGF7* (saliva)

3.3. Accuracy of epigenetic markers regarding gender

As shown in Tables 5 and Figure 2, selected markers showed good sensitivity and specificity. Results suggested that *MT2A*, *LINE-1*, and *FGF7* are good molecular markers for blood and saliva identification regarding gender.

Table 5. Specificity and sensitivity of significant epigenetic markers in blood and saliva identification regarding gender.

Epigenetic markers	**AUC**	**P-value**	**95% Confidence Interval**		**Cut-off value**	**Sensitivity (%)**	**Specificity (%)**
			Lower Bound	**Upper bound**			
MT2A (blood)	0.994	<0.001*	0.978	1.0	1.51	94.1	93.3

LINE-1 (blood)	1.0	<0.001*	1.0	1.0	0.245	100	100
FGF7 (blood)	0.900	<0.001*	0.767	1.0	0.230	82.4	100
MT2A (saliva)	0.902	<0.001*	0.772	1.0	3.85	93.3	88.2
LINE-1 (saliva)	0.996	<0.001*	0.983	1.0	1.56	100	93.3
FGF7 (blood)	0.876	<0.001*	0.755	0.998	0.195	82.4	73.3

AUC is the area under the curve. * Significant at <0.05.

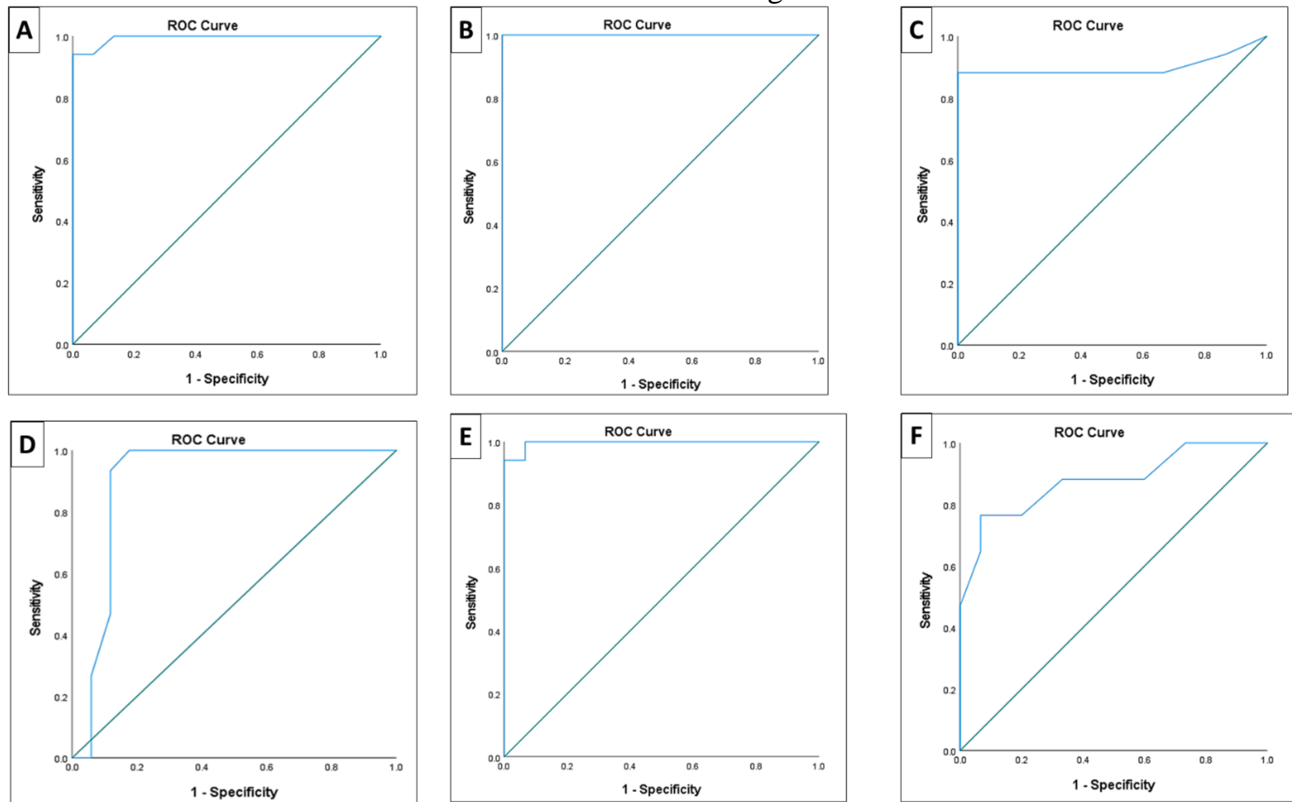

Figure 2. ROC curve for gender identification using: (A) *MT2A* (blood). (B) *LINE-1* (blood). (C) *FGF7* (blood). (D) *MT2A* (saliva). (E) *LINE-1* (saliva). (F) *FGF7* (blood).

4. Discussion

DNA methylation is an attractive marker that can provide investigative leads to solve crimes in forensic genetics. Identifying body fluids that utilize tissue-specific DNA methylation can contribute to solving crimes by predicting the activity that led to evidence material deposition (Lee et al., 2016).

Through this study, the DNA methylation potential was examined to determine body fluids. Four different markers *LINE-1*, *MT2A*, *MGMT* and *FGF7* were selected, and their DNA methylation profiles were obtained. Our results observed differences in the expression of *LINE-1* and *FGF7* markers across nationalities, particularly between Egyptian and Yemeni groups, which offers an intriguing insight into potential genetic or environmental factors influencing these biomarkers. The

statistical significance of these findings, underscores the importance of nationality and possibly underlying genetic predispositions in the expression levels of these markers.

The overexpression of *LINE-1* and *FGF7* in Egyptians compared to Yemenis suggests a differential genetic expression or environmental exposure that could be linked to various health implications or disease susceptibilities unique to these populations. *LINE-1* elements, being retrotransposons, are known for their ability to affect gene expression and genome stability, which could potentially contribute to variations in disease risks or physiological differences between these groups (Paço et al., 2015). Similarly, the role of *FGF7* in cell growth and development, as well as in the repair processes, indicates that its differential expression could have significant implications for tissue regeneration, response to injury, or even carcinogenesis (Luigi et al., 2017).

In addition, Saliva is an attractive alternative for DNA methylation profiling as collection is simple, non-invasive, and high-quality methylation profiles can be generated. For participant recruitment in large research studies, these factors are highly beneficial for increasing participation as seen in a study comparing participation between blood collection (31% positive) and saliva collection (72% positive). This could be extended to a clinical setting where the potential use of saliva to help screen and monitor conditions, may improve compliance rates for patient follow up appointments (Hearn et al., 2019).

The present study detected differences in the expression of *MT2A* and *FGF7* markers in saliva across different nationalities, providing valuable insights into the biological diversity and the potential influence of genetic and environmental factors on gene expression. The significant differences observed for *MT2A* and *FGF7* highlight the role of nationality in the differential expression of these markers, which could have implications for understanding susceptibility to diseases and responses to environmental stresses or treatments.

The overexpression of MT2A in Yemenis compared to Egyptians suggests that there could be genetic or environmental factors unique to the Yemeni population that influence the expression of this metallothionein. *MT2A* is known for its role in metal ion homeostasis and protection against oxidative stress and toxic metals (Zhu et al., 2009), indicating that the Yemeni population may have adaptations or exposures that necessitate enhanced expression of MT2A. This could reflect differences in environmental exposures or dietary habits that necessitate a greater capacity for metal detoxification or oxidative stress response.

Conversely, the differential expression of *FGF7*, with higher levels in Egyptians and Sudanese compared to Yemenis, suggests variations in tissue repair mechanisms or growth factor signaling that could be influenced by genetic factors or environmental conditions. *FGF7* plays a crucial role in epithelial tissue repair and development, and its varied expression could impact wound healing, susceptibility to certain diseases, or responses to environmental damage (Farooq et al., 2021).

Even though DNA methylation profiling is relatively new, it has been previously reported in 1993. The study introduced DNA methylation into forensic sciences by developing a simple procedure for female sex typing based on varying methylation patterns of *DXZ4*, an X chromosome- specific region. The DXZ4 sequence showed low degrees of methylation on the inactive X, but hyper-methylation on the active X chromosomes. The protocol was quite sensitive as due to the high copy number of DXZ4 in the genome, only a minute amount of DNA was necessary for accurate sex typing. Researchers also suggested application for detecting sex- reversed patients (Naito et al., 1993; Vidaki et al., 2013).

In this study, the gender-based differences in the expression of *MT2A*, *LINE-1*, and *FGF7* markers in the blood provided a compelling narrative on the biological distinctions between males and females at the molecular level. The statistical significance of these differences underscores the

profound impact of gender on the expression of these specific markers, suggesting potential gender-specific physiological or pathological mechanisms.

First, the overexpression of *MT2A* in females compared to males highlights a gender-specific response that could be linked to the body's defense mechanism against oxidative stress and heavy metals. MT2A's role in providing cellular protection against environmental stressors may indicate that females have a heightened capacity for detoxification and antioxidant defense, potentially reflecting biological adaptations to ensure reproductive health or differences in hormonal regulation.

Similarly, the higher expression of *LINE-1* in females suggests gender-specific differences in genomic stability and regulation. *LINE-1* elements, being retrotransposons, can influence gene expression and genomic architecture. Their increased expression in females may reflect differences in epigenetic regulation or genome defense mechanisms against transposable elements, possibly influenced by hormonal levels or reproductive factors (Zhang et al., 2020).

Lastly, the significant overexpression of *FGF7* in females further emphasizes the role of gender in influencing gene expression. Given *FGF7*'s involvement in tissue repair and development, its heightened expression in females could suggest a stronger capacity for epithelial tissue regeneration, possibly influenced by estrogen or other factors related to female physiology (Farooq et al., 2021). This difference could have implications for wound healing, skin health, and potential susceptibility to certain diseases.

These findings contribute to the growing body of evidence highlighting the biological differences between genders, extending beyond reproductive functions to encompass various physiological and molecular aspects. Understanding these differences is crucial for developing gender-specific approaches in medicine, including diagnostics, treatment protocols, and drug dosing, to ensure effective and personalized healthcare.

Likewise, the gender-based differences in the expression of *MT2A, LINE-1*, and *FGF7* markers in saliva reveal intriguing insights into how gender influences molecular expressions in a non-blood-based biological matrix. The observation of statistically significant differences for these markers emphasizes the biological variance between males and females at the molecular level, potentially reflecting distinct physiological or pathological processes.

The overexpression of *MT2A* in males compared to females is a notable finding, suggesting a gender-specific adaptation or response mechanism. Given *MT2A*'s role in metal ion homeostasis and protection against oxidative stress, this difference might indicate a higher capacity or necessity for males to detoxify metals and mitigate oxidative stress, possibly due to differences in lifestyle, environmental exposures, or intrinsic biological factors.

Conversely, the increased expression of *LINE-1* and *FGF7* in females suggests significant gender-based molecular differences in genomic regulation and tissue repair mechanisms. The overexpression of *LINE-1* in females could point to a higher activity of transposable elements, influencing genomic stability, gene expression, and possibly the immune response, which hormonal differences or other sex-specific factors might modulate.

Similarly, the higher levels of *FGF7* in females underline the importance of this growth factor in tissue repair and regeneration, possibly reflecting an enhanced capacity for epithelial healing or maintenance in females. This could have implications for skin health, wound recovery, and potentially the response to epithelial damage or diseases, highlighting the need for gender-tailored approaches in medical treatments and interventions.

These findings underscore the complexity of gender differences at the molecular level, extending our understanding beyond the traditionally recognized physiological and anatomical

differences. They highlight the necessity of considering gender as a critical variable in biomedical research, diagnostic procedures, and therapeutic strategies.

The significant disparities in the expression of these markers between genders in saliva, as opposed to blood, also point to the potential for using saliva as a diagnostic fluid for gender-specific studies and personalized medicine. It underscores the relevance of selecting appropriate biological matrices for biomarker discovery and the need for further research to elucidate the mechanisms behind these gender-specific expressions. Understanding these differences is crucial for advancing personalized medicine, ensuring that both diagnostic and therapeutic approaches are optimized for gender-specific physiological and molecular profile.

5. Conclusion

The observed differences in the expression of *LINE-1, MT2A, MGMT*, and *FGF7* markers across nationalities, particularly between Egyptian and Yemeni populations, suggest that genetic or environmental factors may contribute to variations in these biomarkers. These findings underscore the importance of considering population-specific differences in the interpretation and application of DNA methylation profiles in forensic investigations and disease susceptibility studies.

The gender-based distinctions in the expression of *MT2A, LINE-1*, and *FGF7* in both blood and saliva further emphasize the profound impact of biological sex on molecular-level processes. These differences shed light on potential gender-specific physiological and pathological mechanisms, such as oxidative stress response, genomic stability, and tissue repair, which have important implications for the development of personalized diagnostic and therapeutic approaches.

The ability to detect these gender-specific differences in saliva, a non-invasive and easily accessible biofluid, highlights the potential for using this matrix in gender-specific biomarker discovery and clinical applications. This underscores the relevance of selecting appropriate biological samples for biomarker research and the need for further investigations to elucidate the underlying mechanisms behind these gender-specific molecular expressions.

Overall, this study contributes to the growing body of evidence demonstrating the value of DNA methylation profiling in forensic science and personalized medicine. The findings emphasize the importance of considering population-specific and gender-based factors in the interpretation and application of these molecular markers, paving the way for more targeted and individualized approaches in various fields, from crime scene investigations to disease diagnosis and management.

Declarations

- **Consent for publication**
  Not applicable

- **Availability of data and materials**
  All data generated or analyzed during this study are included in this published article.

- **Competing interests**
  The authors declared no competing interests.

- **Funding:**
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- **Authors' contributions**

The first author (Hussein A. Adress) is credited with conceptualizing the study, designing the experiments, analyzing the data, and drafting the initial manuscript. The second author (Dina A. Shokry) is acknowledged for conducting the laboratory experiments, collecting the data, and contributing to data analysis. The third author (Elsayed I. Salim) is recognized for supervising the project, acquiring funding, providing resources, and critically reviewing and revising the manuscript. The final sentence indicates that all three authors reviewed and approved the final version of the manuscript before submission.

All authors have agreed to be personally accountable for their contributions.

And to ensure that any questions about the accuracy or integrity of any part of the work, even if they were not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

The final manuscript was read and approved by all writers.

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