International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Impact of Semen/Vaginal Fluid Mixture on the Expression of miR891a and miR124a in Dried Sexual Stains

Nashwa El-Khazragy[1], Emad A Elsayed[2], Sonya M.S.Azab[3], Assem H.Badway[4], Yaser FAlakid[5], Heba M Halawa[6]

[1]Department of Clinical and Molecular Pathology, Oncology Diagnostic Unit, Faculty of Medicine, Ain Shams University, Cairo, Egypt

[2, 3, 4, 5, 6]Department of Forensic Medicine and Toxicology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Abstract: ***Background: Identification of sexual assault samples is of great importance in the sexual crimes forensic studies. Currently employed routine methods are insufficient. Advances in mRNA markers have been developing and with increasing evidence for the tissue-specificity of microRNAs; short length of 18–23 bp, which overcome the degradation properties of mRNA. Aim of the work: to investigate the impact of mixing semen and vaginal stains on the expression of miR891a and miR124a as biomarkers in dried forensic stains. Methods:MiR-891a and miR-124a expression levels were measured in semen, vaginal fluid stains as well as their mixture using a quantitative Real- time PCR technique.RUN6b was used as an internal reference gene for normalization of targets gene, the sensitivity and detection capability were evaluated. Results:The expression level of miR891a was significantly high in dried semen, although it was undetectable in dried vaginal stains; the same results were detected for miR124a in dried vaginal stains. Both miRNAs targets were detected in dried mixed semen/ vaginal stains with high sensitivity. Conclusions: mixture of semen and vaginal stains doesn't alter miRNAs expression levels with good stability over six month's duration.***

Keywords: Dried sexual stains, miRNAs, Sexual crimes, Semen vaginal mixture, qRT-PCR.

1. Introduction

Sexual assault on women and children are some of the most heinous crimes against Mankind. In scenes of sexual assault crimes, biological evidence such as blood, hair, saliva, semen, vaginal swabs and other body fluid stains are of crucial importance and should be collected [1].Several RNA markers and tDMRs (tissue-specific differentially methylated regions) which are specific to forensically relevant body fluids have been identified and their specificities and sensitivities have been tested using various samples [2, 3].

Recently, micro-RNA markers have started to be explored as an alternative tool for forensic body fluid identification [4]**.** They are non-coding RNA molecules of 18 to 22 nucleotides in length, and regulate gene expression at the post-transcriptional level by binding to the 3′ untranslated regions (3′UTR) of their target mRNAs [5].The miRNAs participate in a variety of regulatory pathways , including cell proliferation, apoptosis, haematopoiesis and so on [6, 7]. ***Hanson's*** [8] proved the ability of miRNAs to be less degraded by environmental factors due to their small size thus offering advantages as ideal molecular marker for body fluid identification.Some reviewers have been published into the identification ofbody fluids using miRNAs; they reported that miRNAs showed tissue specific expression patterns [9, 10]. Several studies have demonstrated that many miRNAs showed tissue-specific expression patterns [9]. Moreover, the intrinsically small size of miRNAs makes them less prone to degradation by environmental factors, thus offering an obvious advantage as a useful biomarker for body fluid identification [8]**.**This particular feature solves problems in old forensic samples identification [11, 12].The study was performed to evaluate theeffect of mix of semen and vaginal swab stains on the expression of miRNAs (miR891a, miR124a) versus non- mixed semen miRNAs in old semen, vaginal stains as well as their mix.

2. Materials and Methods

- **Micro-RNA Selection**

Referred to the high specificity and relative stability of miRNAs as molecular biomarkers used for identification of body fluid samples, the following markers were selected: miRNA-891a (semen), miRNA-124a (vaginal secretion) [8] and both for semen- vaginal stains mix. RNU6b was selected as reference gene (RG) for normalization of expression of miRNA891a and miRNA124a for all samples. The selection was based on the study of ***Saur and his collagenous at 2014*** [13] who reported that RNU6-2 was one of the most stable endogenous reference genes used for normalization of qPCR in forensic samples [13].

- **Samples collection and preparation**

After obtaining the approval from ethical committee for this work, informed consents were obtained from 20 unrelated male volunteers and 20 females with matched ages (25 – 55 years) to participate in the current study. Semen samples were collected by ejaculation in 50 ml falcon tubes considering that (sperm count > 100,000/HPF, with at least 40% motility). Vaginal swabs were collected with sterile cotton swabs. Semen and vaginal swab samples were used to create 20 semen, vaginal and semen/vaginal mix; stains according to the forensic operational processes. The 2ul liquefied semen; was smeared on sterile gauze, natural dried and at 25ºc. The same condition was applied to vaginal fluid stains as well as semen/vaginal fluid mix. Stains were stored at 20º-25ºc under dry and dark conditions. for one day, seven

days, fourteen days, one months, three months and six months. RNA was extracted from all semen, vaginal stains as well as their mix with *miRNeasy human cell/tissues* Kit for forensic specimens" (*Qiagen, Hilden, Germany*), according to manufacturer's instructions. The extracted RNA was quantified by *NanoDrop ND-1000* spectrophotometer (*Thermo Scientific, Wilmington, DE, USA*), then the purified RNA sample was collected in a sterile RNA free 0.2 Eppendorf and stored at -80°C till preparation of cDNA. The primer sequences of the target genes *miR891*, *miR124a*, reference gene *RNU6b* (*miScript primer assay kit*) and the real- time quantitative PCR kit (*miScript SYBR Green PCR kit*) were purchased from (*Qiagen, Hilden, Germany*).

The reverse transcription and RT-qPCR reactions was performed according *to miScriptSyber green assay kit* manual, The expression levels of miR891,miR124a and RUN6b in all samples in addition to negative control were done in duplicates. cDNA were amplified using specific primer sequence.The real time PCR analysis was done using the *StepOne Applied Biosystems (ABI, USA)*. The protocol of for the RT-qPCR components in each tube: miScript SYBR Green PCR Master Mix (2x) 12.5µl, miScript Universal Primer (10x) 2.5 µl, miScript Primer Assay (10x) 2.5 µl, 4.5ul RNase-free water, cDNA product 3 µl. The PCR cycling conditions were (HotStarTaq DNA Polymerase activation; 15 min 95ºC, Cycling; 15 s 94ºC, annealing 30 s and55ºC, extension 30 s 70ºC, for 45 cycles) and the differences in expression levels were compared and expressed as relative expression ($2^{-\Delta Ct}$) or as fold change relative to RNU44 reference gene ($2^{-\Delta\Delta Ct}$). The sensitivity of the test was tested with serial dilution of cDNA (50, 0.5, 0.05, 0.005 ng/ml).To evaluate the impact of time on miRNAs expression values; old samples were analyzed; 1 day, 7 days, 14 days, 1 month, 3 months and six months respectively. Those samples were analyzed by RT-qPCR and C_t values were compared.

- **Statistical analysis**

Analysis of data was performed using Statistical Package for the Social Sciences software (*SPSS,Version 17, Chicago, IL, USA*). Comparisons were performed using analysis of variance (*ANOVA*) test, Pearson's correlation analysis was performed to determine whether there were correlations between the investigated miRNAs in examined body fluid samples. *P* value< 0.05 was considered statistically significant.

3. Results / Discussion

- **miR891, miR124a and RNU6b expression values in semen, vaginal, semen/vaginal mix stain samples:** The amplification curves of miR891, miR124a and RNU6b are obtained at exponential phase when the fluorescence detection threshold was set at 0.06. The C_t value was collected for each sample; the ΔC_t values were calculated as (ΔC_t target gene - ΔC_t reference gene). The results are shown in (table 1). The histogram was created by comparing the relative expression levels (RQ) of miR891a and miR124a in semen, vaginal, semen/vaginal mix (Fig1), the expression levels of miR891a in semen stains was higher than in vaginal stains; miR124a was higher in vaginal stains compared to semen stains; although both were expressed in semen/vaginal mix stains but in lower levels than in primary samples. High significant difference was obtained between miR891in semen stains versus semen/vaginal mix stains ($p<0.002$), similar result was detected in miR124a in vaginal stains. Moreover, high significant difference was detected between miR891a expression levels in semen stains/ vaginal stains as well as miR124a ($p<0.001$). The relative quantitation (RQ) values by $\log^{10}$ presented on y axis. miR891, miR124a is the highest RQ value in semen stains, vaginal swab; respectively. Both miRNAs RQ values decline in semen/vaginalmix stains.

Table 1: The CT and ΔCT values of miR891a and miR124a in different examined stains

Sample		C_T			ΔC_T	
Type	Number	miR891a	miR124a	RUN6b	miR891a	miR124a
Semen stains	SM1	28.42	37.8	36.42	-8	1.38
	SM2	29.66	38.6	37.11	-7.45	1.49
	SM3	29.01	37.05	36.81	-7.8	0.24
	SM4	28.32	38.72	36.24	-7.92	2.48
	SM5	28.99	37.68	36.18	-7.19	1.5
	SM6	28.98	38.29	37.05	-8.07	1.24
	SM7	28.22	37.62	36.51	-8.29	1.11
	SM8	28.02	38.05	36.24	-8.22	1.81
	SM9	29.52	32.27	37.83	-8.31	-5.56
	SA10	28.96	38.86	38.22	-9.26	0.64
	SM11	29.22	37.42	36.41	-7.19	1.01
	SM12	28.72	38.92	38.06	-9.34	0.86
	SM13	27.76	37.95	35.15	-7.39	2.8
	SM15	28.65	38.92	37.52	-8.87	1.4
	SM16	27.89	38.61	36.02	-8.13	2.59
	SM17	27.01	38.68	37.66	-10.65	1.02
	SM18	27.53	38.62	36.06	-8.53	2.56
	SM19	27.88	38.61	37.37	-9.49	1.24
	SM20	28.26	37.37	35.11	-6.85	2.26
Vaginal Fluid Stains	VA1	37.92	28.62	33.28	-4.64	-5.62

	VA2	39.96	29.16	36.9	-3.06	-5.51
	VA3	33.28	28.11	33.5	0.22	-5.76
	VA4	33.31	31.25	34.6	1.29	-5.52
	VA5	34.25	28.37	35.2	0.95	-4.5
	VA6	32.54	29.64	33.4	0.86	-5.76
	VA7	37.11	29.01	38.9	1.79	-5.89
	VA8	32.05	28.37	33.8	1.75	-6.19
	VA9	35.11	28.91	36.6	1.49	-6.56
	VA10	36.24	28.96	37.05	0.81	-8.06
	VA11	33.78	28.05	35.72	1.94	-4.99
	VA12	34.08	28.09	35.68	1.6	-6.84
	VA13	32.98	29.62	33.29	0.31	-4.2
	VA14	32.16	28.69	35.62	3.46	-7.5
	VA15	32.95	29.21	34.05	1.1	-5.41
	VA16	30.81	28.8	31.27	0.46	-7.98
	VA17	34.18	28.71	36.86	2.68	-7.44
	VA18	33.78	27.7	35.42	1.64	-7.76
	VA19	34.68	28.62	36.22	1.54	-4.74
	VA20	32.98	27.58	33.28	0.3	-5.62
	MSV1	38.12	38.28	36.05	-2.07	-2.23
Semen/vaginal fluid mix	MSV2	37.46	37.81	36.42	-1.04	-1.39
	MSV3	36.01	36.85	35.22	-0.79	-1.63
	MSV4	37.95	38.63	35.3	-2.65	-3.33
	MSV5	37.27	38.41	36.9	-0.37	-1.51
	MSV6	36.54	35.6	33.2	-3.34	-2.4
	MSV7	38.11	37.52	36.2	-1.91	-1.32
	MSV8	34.87	34.51	33.1	-1.77	-1.41
	MSV9	32.61	32.68	30.5	-2.11	-2.18
	MSV10	36.26	36.62	35.2	-1.06	-1.42
	MSV11	34.15	35.35	32.9	-1.25	-2.45
	MSV12	36.59	37.56	35.6	-0.99	-1.96
	MSV13	33.22	34.64	32.2	-1.02	-2.44
	MSV14	38.09	37.5	36.14	-1.95	-1.36
	MSV15	36.11	37.22	35.5	-0.61	-1.72
	MSV16	38.6	38.01	37.15	-1.45	-0.86
	MSV17	37.41	38.22	36.4	-1.01	-1.82
	MSV18	36.8	37.46	35.2	-1.6	-2.26
	MSV19	36.12	35.71	34.8	-1.32	-0.91
	MSV20	37.28	37.06	35.6	-1.68	-1.46
Negative Control	NTC	undetermined	undermined	40.22	-----	-----

NTC: negative control; undetermined: RNA is unsuccessfully extracted or very low concentration to be amplified

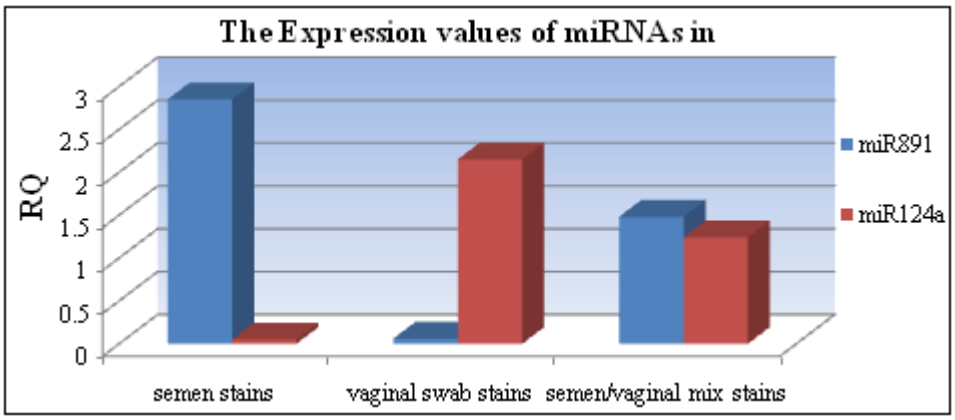

Figure 1: The expression of two miRNAs markers in (semen, vaginal swab, semen/vaginal mix) stains

- **The sensitivity of miR891a and miR124a in identification of semen, vaginal and semen/vaginal stains:** Sensitivity of the method was analyzed by testing miR891a and miR124a in serial dilutions of cDNA. These results were presented in Table 2. Results revealed that to obtain the maximum test sensitivity; cDNA concentration should be > 1 ng/ml.

Table 2: Serial dilutions of RNA to evaluate miRNAs sensitivity by RT-qPCR

Total cDNA (ng)	C_T miR891a	C_T miR124a	RNU6b	ΔC_T [891a-U6]	ΔC_T [124a-U6]	+/-
50	27.01	30.68	38.66	-11.65	-7.98	+
5	29.82	32.27	38.95	-9.13	-6.68	+
0.5	34.61	35.51	undetermined	-------	-------	F
0.05	34.52	38.64	undetermined	-------	-------	F
0.005	32.08	undetermined	undetermined	-------	-------	F
NTC	undetermined	undetermined	undetermined	-------	-------	F

F= failure of amplification of reference gene *RNU6b; this means that RNA is unsuccessfully extracted or very low concentration to be amplified, NTC= negative control

The reliability of miR891a and miR124a in identification of old samples: Stains stored at different times results were statically analyzed; no significant differences were detected at different test intervals up to six months in semen, vaginal stains (p=0.08); However, statically difference was found between semen/vaginal mix stains in old samples (p=0.03). Therefore, the results suggest that the method is reliable (Table 3, Fig 2).

Detection of degraded or old forensic samples is a problematic issue; therefore many researchers tried to find out a solution [8, 11, 12, 14]. The discovery of miRNAs help to solve this problem; miRNAs are short fragments which are slowly degraded compared to mRNA, their molecular characteristics and tissue specificity make them a suitable molecular markers for identifying body fluid stains in forensics [15-16].The results of this study demonstrate that the expression level of miR891a was higher in semen stained samples than that of reference gene RUN6b.

Table 3: miRNAs expression results in stored stains for six months

Storage time at room temperature	Semen stain		Vaginal swab stains		Semen/Vaginal mix	
	No of samples	Positive samples	No of samples	Positive samples	No of samples	Positive samples
1 day	20	20	20	20	20	20
7 days	20	20	20	20	20	20
14 days	20	20	20	20	20	18
1 month	20	18	20	19	20	17
3 month	20	18	20	18	20	16
6 month	20	18	20	18	20	15

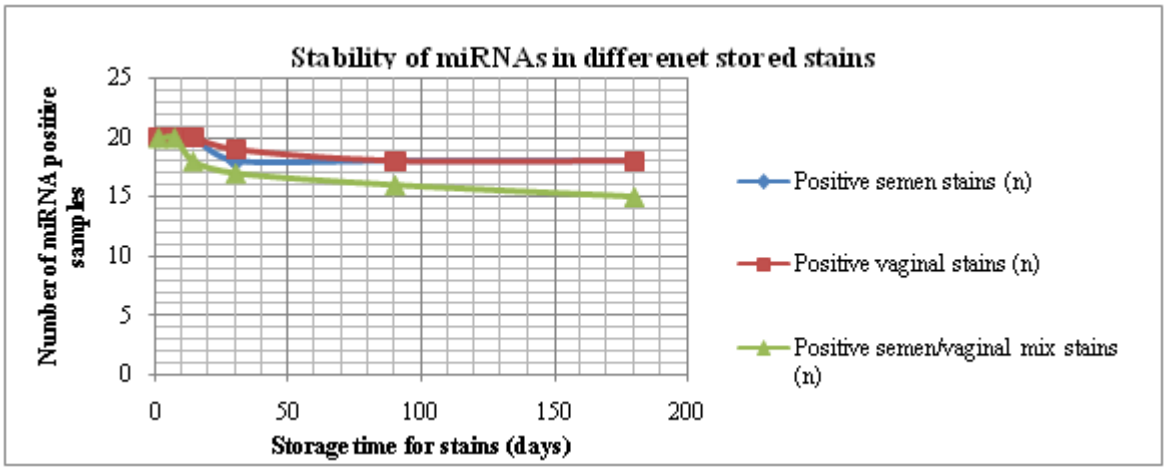

Figure 2: The smooth line scatters with marks. The number of miRNAs positive samples in (semen, vaginal swabs, semen/vaginal mix); stored stains at room temperature for 180 days are presented on Y axis. Semen stains are the most stable followed by vaginal swabs and semen/vaginal mix stains.

Similar results were detected for miR124a in vaginal stains. Statistical analysis of the expression levels of both targets in semen and vaginal stains; it was found that these miRNAs can distinguish semen from vaginal stains. The standard criteria used were as follows: the C_t value of target genes miR891a, miR124a and reference gene RUN6b are <40 when the crossing threshold value was set at 0.06, the ΔC_t of miR891<-6.85 in semen stains and ΔC_t of miR124a <-4.7 in vaginal stains to distinguish semen from vaginal stains. In this study, 20 semen and vaginal stains were analyzed for both targets. When these results were compared to standard criteria, all semen, vaginal stains were above the cut off value of miR891a and miR124a respectively. To confirm the specificity of miR891a in identifying semen samples, five semen samples with azoospermia were analyzed using the same method and criteria; the results fall within the positive range. In the same way 20 vaginal stains were analyzed; only two samples out of 20 were positive. Similar results

were found for miR124a, all vaginal stains were positive; however, miR124a expression levels in semen samples were below the detectable cut off value except one sample which fall within the positive range (Table 1). The results of the two vaginal stains, semen stain were positive for miR891a and miR124a respectively were confusing and the reasons were complicated. Explanation of these results may be related to technical error, abnormality of the samples such as an influence of unknown disease altering these miRNAs expression levels. So, further researches are required to study this point. Therefore, miR891 can distinguish normal as well as azoospermic semen stains from vaginal stains in forensic sexual assault samples. Same findings could be applied on vaginal / semen stains by using miR124a as molecular marker. Similar findings were reported by previous studies that evidenced specificity of miR-891a to semen as ***Sauer's***[17] **[**whostated that miR-891a is a reliable biomarker for semen identification.Also,***Zubakov***[5]profiled the expression levels of 718 miRNAs in menstrual blood, venous blood, saliva, semen and vaginal secretion on a micro-array and identified 14 differentially expressed candidates for potential body-fluid identification. They found both of miR-135a and miR-891a as the most abundant and most specifically expressed in semen. These findings are in agreement with previous studies that reported specific tissue-expression pattern of miRNAs. ***Wang's*** [18]employed the qPCR-array to screen the body fluid-specific miRNAs and identified seven candidate miRNAs as potentially body fluid-specific: miR-16 and miR-486 for venous blood, miR-888 and miR-891a for semen, miR214 for menstrual blood, miR-124a for vaginal secretions, and miR-138-2 for saliva. This is in accordance with ***Landgraf***[19]who reported that, many miRNAs are expressed in a tissue-cell-specific manner. They found expression of miR-891a only in epididymis tissue while it was practically absent from any other tissue analyzed. We also agrees with other studies that revealed specificity of miR-124a to vaginal secretions;***Hanson's***[8]and ***Bai*** [21]who studiedmiRNA expression in dried, forensically relevant biological fluids (blood, semen, saliva, vaginal secretions, and menstrual blood). They identified a panel of nine miRNAs that are differentially expressed, they reported that miR-124a, and miR-372 can be used as miRNA markers in differentiation of vaginal stains from other body fluids.

Contrary to the current study, ***Luo's***[22]screened fourteen microRNA markers for peripheral blood, menstrual blood, saliva, vaginal secretion, semen and skin cells using microRNA array covering all human microRNAs. They selected miR-3134 and miR-31-3p as markers of vaginal secretions and miR-888-5p miR-135a-5p as markers of seminal stains since they showed specific patterns for these body fluids in the results of array. However, they recommended further confirmation of these markers through RT-qPCR.

To our knowledge, we are the first who studied the effect of mix semen/vaginal samples on the expression levels of miRNAs and compare it with that in non-mixed samples. In this study, we try to find out a solution for problems facing identification of mixed forensic samples, therefore, 20 mixed semen-vaginal stains were analyzed for the targets miRNAs (miR891a and miR124a) and results were compared to their expression levels in non-mixed stains. The ΔC_t for miR891a and miR124a in mixed stains were $\leq$ -0.37 and $\leq$ -0.86 respectively. Similar findings were reported by***Ballantyne's***[23]who found that, vaginal secretions did not affect the detection of semen specific microRNA in vaginal and cervico-vaginal swabs collected from a female donor 18 hours post-coitus.This agrees also with***Hanson's***[24]since he studied miRNA expression profile in body fluid mixtures samples, including blood-semen and semen-saliva mixtures. He could identify all studied body fluids correctly in all mixtures through miRNAs biomarkers. The current study is in accordance with ***Courts and Madea***[25]who found that miRNA assays could detect components in mixtures of liquid blood and liquid saliva. Furthermore, they found all studied miRNAs showed strong expression comparable to their values in isolated body fluid (blood or saliva). ***Uchimoto's***[12]found that, microRNA based body fluid identification tests were capable to differentiate between blood and saliva in their mixture with a 100% success rate. They concluded that the miRNA -based body fluid identification tests can be used on mixed body fluid samples with reliable results.This finding can be attributed to the effect of pH changes related to mixture; pH alternation can protect the mRNA from degradation, keep it more stable. **Fordyce's** [26]foundthat alkali conditions, including the levels naturally found inside cells increase the susceptibility of RNA to hydrolysis, so mixed stain may have approximately neutral PH. So alkalinity of pH decreased with subsequent decrease in hydrolysis of RNA. In addition, this may be due to binding of miRNAs with proteins in mixed stains that inhibit their degradation and prolong their half-life [25].

In this study, old samples were analyzed for miR891a and miR124a after one day, two days, seven days, fourteen days, one month and six months at 25ºC (under dry and dark conditions), the results demonstrated that the positive rates in semen and vaginal stains, semen-vaginal mix stains were 90% , 75% respectively after six month preservation. Therefore, it was concluded from these results that miRNAs markers are stable and can be used for identification of old samples. Our results were matched with [27] who found no significant differences between miRNA-891a expressions in semen samples between aged (after one year) and initial analysed samples. The aged samples were stored under lab conditions (relatively constant humidity and ambient temperature, no UV exposure, dust free, etc.). However, ***Zheng Wang's*** [27] study, revealed that not only miRNAscould be easily detected in experimentally aged body fluid samples but also that the absolute levels do not seem to be diminished in old samples. Similar findings regarding miRNAs stability were reported by ***Zubakov's*** [5]**,** ***Courts's and Madea's*** [25] who mentioned that miRNAs was stable for one year-old blood stains sample, although, they didn't study the stability of miRNAs in semen or vaginal secretion material.

4. Conclusion

In this study, miR-891a, miR-124a exhibited stability from seminal fluid, vaginal material respectively as well as their mixtures. So, the current study approved that miRNAs (891a, 124a) can be used in combination for identification of

forensic samples in sexual crimes. We found that both studied miRNAs markers were stable practically not prone to degradation in old stains up to six months duration. We also confirmed that the respective Real time-PCR assays used here for their detection were highly sensitive, allowing the reliable marker detection from subpicogram amounts of total RNA.

5. Future Scope

While current forensic mRNA profiling allows personal identification of biological sample donors, recent advances in forensics has suggested using other types of markers in order to add more informative layers to the evidence. mRNAs markers have been most rigorously investigated and the number of specific markers is sufficient for the identification of forensically relevant body fluids,but in most cases, tissue specificity is problematic due to cross reaction with biological or non-biological material present in samples at crimes scene. On the other hand, miRNAs markers show high tissue-specificity and adequate sensitivity for forensic analysis due to their stability, thereby being considered as a valuable new approach to overcome the limitations of conventional methods. miRNAs expression still needs to identify more markers for future practical application to casework. We believe that it is now possible to identify more informative markers for body fluid identification using various high-throughput screening technologies. In the near future; forensic investigations should improve a great deal because of the continued advances in genetics, epigenetics and molecular biology, and hence, the extraction of more information from forensically relevant biological samples will be possible. Also,it is crucial to test whether the expression of candidate miRNAs for body fluid identification may be influenced by biological processes or conditions such as cancer, mix with other biological samples rather than tissue type identification.

Funding

This work was supported by the Ain Shams Faculty of Medicine Grants office, (Grant no: 2016/28).

References

[1] California Department of Justice, (2002):Physical evidence Bulletin. Available at: http://www.crime scenin vestigator. net/ CA bloodfordna.pdf.
[2] An JH, Choi A, Shin KJ, Yang WI and Lee HY. (2013).DNA methylation-specific multiplex assays for body fluid identification. Int. J. Legal Med, 127, 35–43.
[3] An JK, Shin K-J, Yang WI and Lee HY. (2012. Body fluid identification in forensics. BMB Reports, 45, 545-553.
[4] Wang, Z., Luo, H. B., Pan, X. F., Liao, M. and Hou, Y. P A. (2012).Model for Data analysis of microRNA expression in forensic body fluid identification. Forensic Sci. Int. Genet, 6, 419-423.
[5] D. Zubakov, A.W. Boersma, Y. Choi, P.F. van Kuijk, E.A. Wiemer, M. Kayser. (2010). MicroRNA markers for forensic body fluid identification obtained from microarray screening and quantitative RT-PCR confirmation. Int. J. Legal Med, 124, 217-225.
[6] Beuvink I, Kolb FA, Budach W, Garnier A, Lange J, Natt F, et al. (2007). A novel microarray approach reveals new tissue-specific signatures of known and predicted mammalian microRNAs. Nucleic Acids Res, 35, e52.
[7] Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ. (2008) MiRBase: tools for microRNA genomics.Nucleic Acids Res, 36, D154–D158.
[8] E.K. Hanson, H. Lubenow, J. Ballantyne. (2009).Identification of forensically relevant body fluids using a panel of differentially expressed microRNAs, Anal. Biochem, 387, 303–314.
[9] Liang Y, Ridzon D, Wong L and Chen C. (2007). Characterization of microRNA expression profiles in normal human tissues. BMC Genomics, 8-166. Available at: http://bmcgenomics.biomedcentral.com/ articles/10.1186/1471-2164-8-166.
[10] Van der Meer, D.et al. Donny van der Meer,1 B.Sc.; Mari L. Uchimoto,2 M.Sci.; and Graham Williams,2 Ph.D.(2013). Simultaneous Analysis of Micro-RNA and DNA for Determining the Body Fluid Origin of DNA Profiles. J Forensic Sci, 58, 1556-4029.
[11] Virkler K, Lednev IK. (2009). Analysis of body fluids for forensic purposes: from laboratory testing to nondestructive rapid confirmatory identification at a crime scene. Forensic Sci, 188, 1–17.
[12] Uchimoto ML, Beasley E, Coult N, Omelia EJ, World D, Williams G.(2013). Considering the effect of stem-loop reverse transcription and real-time PCR analysis of blood and saliva specific microRNA markers upon mixed body fluid stains. Forensic SciInt Genet,7, 418–421. doi: 10.1016/j.fsigen.2013.04.006 PMID: 23768313.
[13] Sauer E, babion I, Madea B, Court C. (2014). An evidence based strategy for normalization of quantitative PCR data from miRNA expression analysis in forensic organ tissue identification. Forensic Science International Genetics, 13, 217–223.
[14] Gao LL, Li YY, Yan JW, Liu YC. (2011). Application and progress of RNA in forensic science. Fa Yi XueZaZhi, 27,455–459. PMID: 22393599.
[15] Zhu J, Feng X, Lou J, Li W, Li S, Zhu H, et al.(2013). Accurate Quantification of microRNA via Single Strand Displacement Reaction on DNA Origami Motif. PLoS One, 8, e69856. doi: 10.1371/journal.pone.0069856 PMID: 23990889
[16] Hanson EK, Ballantyne J. (2013). Circulating microRNA for the identification of forensically relevant body fluids. Methods MolBiol,1024, 221–234. doi: 10.1007/978-1-62703-453-1_18 PMID: 23719955.
[17] Sauer E, Reinke A and Courts C, (2015): Validation of forensic body fluid identification based on empirically normalized miRNA expression data. Forensic Science International: Genetics Supplement Series, 5, e462–e464.
[18] Wang Z, Zhang J, Luo H, Ye Y, Yan J and Hou Y. (2013). Screening and confirmation of microRNA markers for forensic body fluid identification.Forensic Science International.Genetics, 7,116-123.

[19] Weber, J.A., et al. (2010).The MicroRNA Spectrum in 12 Body Fluids.Clinical Chemistry. 56(11): p. 1733-1741.
[20] Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, et al (2007). A mammalian microRNA expression atlas based on small RNA library sequencing. Cell, 129, 1401–1414.
[21] Bai P, Deng W, Wang L, Long B, Liu K, Liang W and Zhang L.(2013). Micro RNA profiling for the detection and differentiation of body fluids in forensic stain analysis.Forensic Science International Genetics, 4, e216-e217.
[22] Luo XY, Li ZL, Peng D, Wang L, Zhang L and Liang WB.(2015). MicroRNA markers for forensic body fluid identification obtained from miRCURY™ LNA array. Forensic Science International: Genetics, 5, e630–e632.
[23] Ballantyne J, Hanson E and Lubenow H. (2015). Method for determining the origin of a sample. University of Central Florida Libraries, p:335.Available at.http://stars.library.ucf.edu/cgi/viewcontent.cgi?article=1334&context=patens
[24] Hanson EK. (2008).The development of a "genetic eyewitness" profiling system for low template forensic specimen's identification of novel protein, RNA and DNA biomarkers. Thesis, PhD in biomolecular Science , Department of Chemistry, Burnett School of Biomedical Sciences, College of Medicine University of Central Florida Orlando, Florida ,USA. Available at: http://etd.fcla.edu/CF/CFE0002373/Hanson_Erin_K_20 0812_PhD.
[25] Courts C and Madea B. (2010). Micro-RNA–A potential for forensic science?Forensic Science International, 203,106-111.
[26] Fordyce S, Marie-Louise Kampmann M-L, Doorn NLV and Gilbert MTP. (2013). Long-term RNA persistence in post-mortem contexts. Investigative Genetics, 4, 1-7.
[27] Zheng Wang,1 Ph.D.; Ji Zhang,1 Ph.D.; Wei Wei,1 Ph.D.; Di Zhou,1 M.D.; Haibo Luo,1 Ph.D.; Xiaogang Chen,1,2 Ph.D.; and Yiping Hou,1 Ph.D.(2015). Identification of Saliva Using MicroRNA. J Forensic Sci, doi: 10.1111/1556-4029.

Author Profile

Nashwa El-Khazragy,Lecturer of Clinical/Molecular Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Emad El –Sayed, Assistant Lecturer, Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Ain Shams University.

Sonya Azab,Assistant Professor, Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Ain Shams University.

AssemH.Badawy, Professor,Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Ain Shams University.

Yaser F Alakid, Professor, Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Ain Shams University.

Heba M Halawa, Professor, Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Ain Shams University.