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Research Article

Blood and Saliva Identification through miRNA in Forensic Molecular Biology

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Abstract

A class of non-coding miRNAs has attracted a lot of interest in the field of forensic sciences. miRNA has a relatively small size thus it is quite stable to the external environmental pressures and factors, which makes it very useful in forensic examination when used as a bio marker. At present, many of the specific miRNAs of body fluids can be distinguished by measuring their level of expression. Fresh blood samples and saliva samples were taken from 3 males and 3 females. RNA was extracted using TRIzol method and cDNA synthesis was done. RT reactions were made using SYBR Green master mix for RT-qPCR for quantification and detection of miRNAs. The designed primers were runs against the sample in qPCR and result was seen and compiled. Two miRNA were detected and expressed in both blood and saliva but they failed to differentiate between blood and saliva due to the non-specific primer binding according the methodology used in this study. Hsa-miR20a and Hsa-miR583 have been found to be non-specific for the blood and saliva. No stark difference could be observed on the basis of which we can say that Hsa-miR20a and Hsa-miR583 were specific for blood and saliva due to non-specific primer binding. Further research work is required in this domain of body fluids identification and differentiation using miRNA markers.

Keywords: Forensics; miRNA; TRIzol; RT-qPCR; Blood; Saliva; SYBR Green; Identification; Sample; cDNA

Abbreviations

RT-qPCR: Reverse Transcriptase Quantitative Polymerase Chain Reaction; miRNA: MicroRNA; qPCR: Quantitative Polymerase Chain Reaction; BFI: Body Fluid Identification; cDNA: Complimentary DNA

Introduction

Forensic science, in its broadest sense can be said as the science that applies scientific analysis to aid the criminal justice system, often helping to prove the incidence of crime, exonerating innocent and even identifying and punishing the culprit. Forensic scientists analyze and interpret the evidence found at crime scenes. This evidence may include blood, saliva, fiber, tire tracks, drugs, alcohol, paint chips, and firearm residues [1]. microRNAs due to their small size and stability have had an increased interest in the field of forensic body fluid identification. miRNAs regulate gene regulation through degradation of mRNA or by halting and stopping protein synthesis. Recently, many studies have been conducted using these miRNAs and a lot of these studies have shown that miRNAs have an important role as a biomarker of disease in the morphology, as well as their role in forensic sciences which include body fluid identification and detection of specific body fluids in addition to being a tool for understanding gene regulation [2].

Many studies have been performed on the efficacy of miRNA as a marker for body fluid identification. Zubakov et al. used 14 primers for detection of four different body fluids. The authors of this study found that the markers for saliva that they used including the marker Hsa-miR583, did not show good expression levels with the method that they used. The expression levels of the markers for blood including the one which we used in this study showed good expression levels according to their data. It has to be noted that they used the Taqman instead of SYBR Green [3].

To further examine the role of miRNAs in body fluid identification, a study was performed by Wang. Z et al., the purpose was to develop an accurate and reliable methodology for analyzing miRNA expression levels and related data. Results showed that the blood taken from vein was isolated from other body fluids used in this study while the expression level of miR658 was not stable and was varying between different body fluids. The results, all in all, can be said that they showed miRNAs are promising in forensic body fluid identification [4].

In another study, the author examined and developed a strategy to define saliva using a miRNA biomarker for forensic purpose. They conclude that no primers were saliva specific. They also noted that some of them primers showed low concentration level. A new strategy was developed in which they concluded that three groups of miRNAs can distinguish between saliva and other body fluids [5].

In the current study, two miRNA markers namely hsa-miR20a for blood and hsa-miR583 for identification of saliva have been used. SYBR Green master mix was used with the method that was developed and the results were compiled, observed and analyzed.

Materials and Methods

The following flow chart, (Figure 1) shows the methodology used

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in this study.

Blood sample from each individual was collected in EDTA vial and saliva sample from each individual was collected in Eppendorf tubes. In this research, blood and saliva samples were used to extract RNA using TRIzol Method. Single stranded cDNA synthesis was done by using Reverse Transcriptase Kit, Thermo Scientific Catalogue # K1622. Primer was designed by using PrimerBlast. The RT-qPCR was performed on Rotor Gene Q qPCR system by Qiagen. SYBER Green Master Mix (WizPure, catalogue # W1711) was used for quantification.

Results

Two miRNA were detected and expressed in both blood and saliva but they failed to differentiate between blood and saliva due to the non-specific primer binding according the methodology used in this study.

Overall it can be seen that saliva samples showed better expression then the blood samples which were used in this study.

Figure 2 demonstrates the standard bar graph of blood samples with blood specific miRNA primer.

Figure 3 demonstrates the standard bar graph of saliva samples with saliva specific miRNA primer.

Blood and saliva were detected using miRNA markers but these two miRNAs were not body fluids specific so we can't differentiate between blood and saliva using these two miRNAs because they are

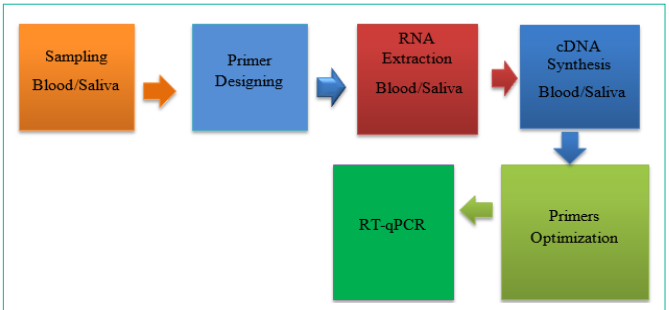

Figure 1: The methodology used in this study.

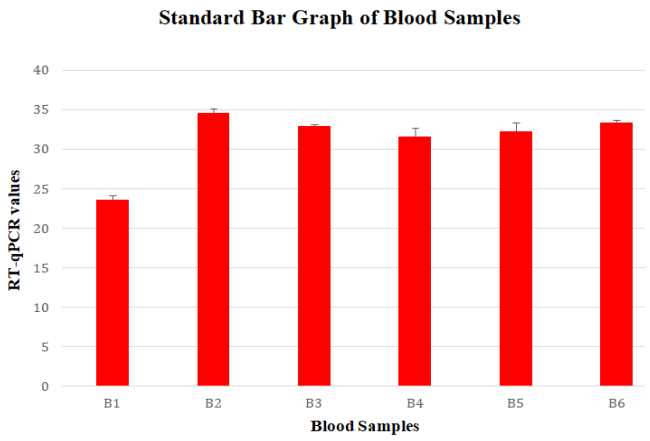

Figure 2: The standard bar graph of blood samples with blood specific miRNA.

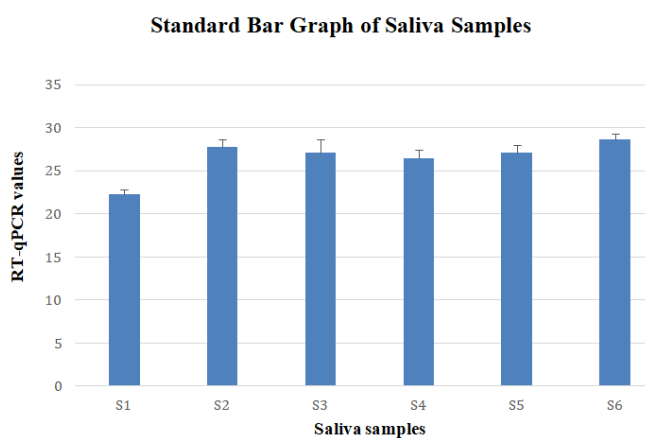

Figure 3: The standard bar graph of saliva samples with saliva specific miRNA primer.

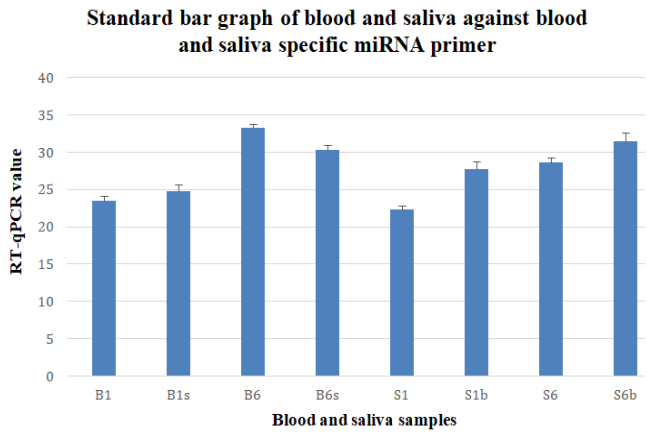

Figure 4: The standard bar graph demonstrates the comparison of blood samples against blood specific miRNA primer with blood samples against saliva specific miRNA primer also saliva samples against saliva specific miRNA primer with saliva samples against blood specific miRNA.

failed to differentiate between blood and saliva due to non-specific primer binding. One possible reason is the difference in the detection method was that used. We used traditional method of RNA extraction with TRIzol method, cDNA synthesis kit and SYBER green master mix for RT-qPCR. Our results showed differences with Zubakov et al., The difference maybe because of using a different qPCR method. Some previous studies used a stem-loop primer qPCR method which has a higher specificity for targeted miRNA than ours. In our knowledge very rare or no studies have explored the incongruity in miRNA detection between methods.

Figure 4 shows the standard bar graph demonstrate the comparison of blood samples against blood specific miRNA primer with blood samples against saliva specific miRNA primer also saliva samples against saliva specific miRNA primer with saliva samples against blood specific miRNA primer.

Discussion

The above results indicate that the samples of the body fluids used in this experiment namely, blood and saliva were detected using the both the primers which were used in the study. The blood primer

Hsa-miR20a and the primer selected for saliva Hsa-miR583, both were able to detect the body fluids be it blood or saliva. The blood specific primer detected all the samples of blood and saliva as well. The saliva specific primer detected all the samples of saliva and blood as well. It has to be noted that even though both the primers were able to detect both the body fluids but it was seen that the expression levels for the body fluids using these two primers were quite similar. No such stark difference could be observed on the basis of which we can say that these primers were specific for each of the body fluids. The expression levels of the blood primer against the saliva was very much the same as the expression levels of blood primer was against the blood sample. Similarly, the same behavior was noted when the saliva primer was run against saliva and the blood samples. Although the expression level of saliva primer were lower against saliva samples than the expression level which were obtained using the blood primer with blood samples in the light of which we can say that the expression levels were better of the saliva primer against saliva than the blood samples against the blood. Similar expression levels, with no stark difference indicate low specificity for both the primers against blood and saliva samples both thus making the differentiation between the two body fluids using these two primers not possible. The non-specificity can be due to various reasons which can include the methodology which was used in this study, the dye might also be non-specific or it can also be said that primers which we have used in this study, for blood and saliva both, these primers were non-specific in nature.

Our study showed non specificity using the SYBR Green using the method we used. It may be due to the locked nucleic acid (LNA) technology which was used in a previous study by Dunnett et al., which increased the specificity of the SYBR Green using the same method. The study by Dunnett et al., used both the traditional method with the addition of LNA technology and the Stem loop method. The authors mentioned disadvantages and the advantages of the both methods they used.

The method we used for our study limited the target RNA variability as suggested in the study by Dunnett et al., In addition to that the reason of using this method was that it was easily accessible in the facilities in Pakistan and such studies have not been performed in Pakistan in which this method has been validated using these two markers. The advantages of the method we used are that we can get a complete reaction with the whole RNA thus it decreases the sensitivity of the RNA which however, has a disadvantage of its own which is that when the sensitivity decreases, the specificity can also decrease drastically, which we can assume has happened in our study as well. However, the stem loop method though, is very rare and not commonly available in the facilities in Pakistan. If we used the stem loop method, we would have to change the whole methodology from the start for RT reaction. However, it was seen in the earlier studies that the stem loop method does have higher specificity for targeted miRNA [6]. However, there are chances of RNA loss and as it only acts on target miRNA regions it may not be as useful for forensic investigations as the conventional method that we have used in our study, according to our observation as normally we may find very small quantity of the body fluids at the crime scenes and in such situations the depletion of the RNA quantity may not be a viable option and as we know that the RNA quantity reduces after quantification, this method may not be as useful as the method we have used in our study.

From the results of our study, we can conclude that the two primers we used, one for the blood and the other for the saliva both were non-specific but both the markers were able to detect the body fluids but not able to differentiate between them. We can conclude these markers we used are not specific to body fluids using this methodology. Further studies on the saliva and blood specific markers used in this study are required using more advanced methods and different techniques and methodologies other than the ones used in this study. We can also improve upon this study by using different markers using the same methodology and validate the results of this study. In the light of the results of this study, we can safely conclude that the markers we used for blood and saliva were not able to differentiate between the two body fluids that were studied in this experiment although both the markers did detect the body fluids but did not differentiate or tell that which fluid is which. Furthermore, there are plenty of researches to be conducted in this domain as this research covers body fluids identification and its novelty stands as this the first research that is conducted in this domain, in Pakistan.

Conclusion

Blood and saliva were detected using miRNA markers but these two miRNAs were not body fluids specific so we can't differentiate between blood and saliva using these two miRNAs because they are failed to differentiate between blood and saliva due to non-specific primer binding. One possible reason is the difference in the detection method was that used. We used traditional method of RNA extraction with trizol method, cDNA synthesis kit and SYBER green master mix for RT-qPCR. Our results showed differences with Zubakov et al., The difference maybe because of using a different qPCR method. Some previous studies used a stem-loop primer qPCR method which has a higher specificity for targeted miRNA than ours. In our knowledge very rare or no studies have explored the incongruity in miRNA detection between methods. Furthermore, there are plenty of researches to be conducted in this domain as this research covers body fluids identification and its novelty stands as this the first research that is conducted in this domain, in Pakistan.

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