ROLE OF AGING IN IDENTIFICATION OF DNA EXTRACTED FROM BLOOD AND SEMINAL STAINS

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ABSTRACT

DNA investigation plays a vital role than ever before in criminal cases as samples retrieved from crime scene may be exposed to different conditions before proceeding that can lead to degradation of DNA.One of these conditions is the aging effect. The aim of this study was to evaluate the effect of aging process on DNA extraction and typing from blood and seminal stains.The present study was done on twenty male volunteers after written consent; four ml of blood and two ml of semen were collected from the same volunteer and spotted on pieces of cotton fabric. The effect of aging (one, three and six months) extraction of DNA from blood and seminal stains were tested. DNA concentration was measured by spectrophotometer and analyzed by using gel electrophoresis. It was shown that blood and seminal stains left at room temperature for one, three and six months can be identified using presumptive tests. DNA concentration was significantly reduced in blood and seminal stains left for one, three and six months before processing. Quality of DNA was not affected by storage length and DNA fragments could be identified except for bloodstains left for six months which cannot detected by Amelogenin primer. In conclusion; DNA can be extracted from blood and seminal stains left for one, three and six months in room temperature. It is recommended to expose the samples to longer periods more than 6 months at room temperature. Different primers can be used for further research. Other types of samples can be used in further studies rather than blood and semen as saliva and teeth.

Key word: blood–semen—degradation – DNA – Amelogenin – primers.

I-   INTRODUCTION

DNA analysis plays a vital role in crime scene investigations, both to convict the guilty or exonerate the mistakenly accused. DNA is also playing an important role in identification of victims in mass disaster and crimes (Jobling and Gill, 2004).

It is a potent investigative tool because there are not two persons have the same DNA, with the exclusion of identical twins. In other words, the sequence of the DNA building blocks is altered in particular regions of the cell, making each individual's DNA unique (Hattori, 2005).

Bloodstains can be found anywhere in the crime scene. One of the main tasks of the forensic expert in crime scene is to examine the materials left. The increase in the number of crimes; makes the need for studying the effects of environmental factors that affect the sample is so important, in order to stop the degradation of DNA (Bittencourt et al., 2009).

Seminal stains left at a crime scene are also important evidence in many types of crimes, including sexual crimes (Nakanishi et al., 2014).

If crimes remain unsolved for long periods, body fluid and bloodstains can be re-investigated using DNA typing methods such as short tandem repeat (STR) typing (Hara et al., 2015).

This work sheds light on the effect of different aging periods on the quantity and quality of DNA, retrieved from blood and seminal stains.

II-   SUBJECTS AND METHODS

The present study was conducted from the 1st of March 2018 to the 30th of December 2018. Samples (blood and semen) were collected from twenty male volunteers after taking informed written consent; all of them were patients in Andrology clinic, Assiut University Hospitals. The samples were processed in the Forensic Medicine and Clinical Toxicology department, Faculty of Medicine, Assiut University and Molecular biology unit, Assiut University.

Ethical approval:

All ethical statements of The Research Ethics Committee, Faculty of Medicine, Assiut University, were followed. All participants were filled an informed consent after full explanation of the study and its aim. No risk for volunteers if they refused to share in the study. The ethical approval number is 17200332.

Inclusion criteria:

Age group was from 20-40 years old (Singh et al., 2003).

Exclusion criteria:

There are no any exclusion criteria as STR core loci are not known to be affected by any medical conditions (Hares, 2012).

1- Sample processing:

A- Blood and semen samples collection:

Four ml of blood and two ml of semen were taken from each volunteer. Blood samples were taken by venipuncture and semen samples were taken by masturbation. The blood samples were collected in two vacutainer K2E EDTA tubes and semen samples were collected in plastic sterile containers. The blood and semen samples were assigned codes to protect the privacy of the donors and allow for unbiased evaluation (Somiari et al., 2011).

B- Blood and semen Sample Processing;

Each blood sample was divided into four parts, one ml each, spotted on four pieces of autoclaved white cotton fabric of surface area 3×3 cm (Antrim et al., 2004). The cotton fabric was tested as a negative control for blood DNA without the addition of blood samples. They were left over night to **II-** dry on laboratory counter after their sterilization with ethyl alcohol with ambient room temperature in Assiut governorate (Max 26.7˚c) (Ali, 2012).

Each semen sample was divided into four equal parts, half ml each, by dropper and spotted on four pieces of autoclaved white cotton fabric of surface area 3×3 cm. The cotton fabric was tested as a negative control for blood DNA without the addition of semen samples. The cotton fabric pieces containing the seminal stain were left over night to dry on laboratory counter after their sterilization with ethyl alcohol with ambient room

temperature in Assiut governorate (Max 26.7˚c) (Ali, 2012).

C- Grouping :

I- Blood samples (B) were divided into the following:

1- **Group (1) :** 20 samples assigned the code B* and were considered as The positive control which processed immediately after drying

2- **Group (2):** 20 samples assigned the code B(f). Blood samples from each volunteer were put in paper envelope separately and left in room temperature (in the closet) for one month (from the 1st to the 31st of March) before processing with ambient room temperature (Max 26.7˚c) (Ali, 2012) .

3- **Group (3)**: 20 samples assigned the code B (g). Blood samples from each volunteer were put in paper envelope separately and left in room temperature (in the closet) for three months (from the 1st of March to the 31st of May) before processing with ambient room temperature in March (Max 26.7˚c), April (Max 31.8˚c) and May (Max. 35.8˚c) (Ali, 2012).

4- **Group (4):** 20 samples assigned the code B (h). Blood samples from each volunteer were put in paper envelope separately and left in room temperature (in the closet) for six months (from the 1st of March to the 31st of August) before processing with ambient room temperature in March (Max 26.7˚c), April (Max 31.8˚c), May (Max. 35.8˚c), June (Max 37.2˚c), July (Max. 37.2˚C) and August (Max 36.9˚c)(Ali, 2012).

Semen samples (S) were divided into:

1- **Group (1) :** 20 samples assigned the code S(*). The positive control which processed immediately after drying.

2- **Group (2) :** 20 samples assigned the code S(f). Seminal samples from each volunteer were put in paper envelopes separately and left in room temperature (in the closet) for one month (from the 1st to the 31st of March) before processing with ambient room temperature (Max 26.7˚c) (Ali, 2012) .

3- **Group (3):** 20 samples assigned the code S(g).Seminal samples from each volunteer were put in paper envelopes separately and left in room temperature (in the closet) for three months (from the 1st of March to the 31st

of May) before processing with ambient room temperature in March (Max 26.7°c), April (Max 31.8°c) and May (Max. 35.8°c) (Ali, 2012).

4- **Group (4) :** 20 samples assigned the code S(h).Seminal samples from each volunteer were put in paper envelopes separately and left in room temperature (in the closet) for six months (from the 1st of March to the 31st of August) before processing with ambient room temperature in March (Max 26.7°c), April (Max 31.8°c), May (Max. 35.8°c)**,** June (Max 37.2°c), July (Max. 37.2°C) and August (Max 36.9°c) (Ali, 2012).

2- Examination of the samples:

A- Macroscopic examination of blood and semen samples:-

Each sample was examined by naked eye and photographed for the visual identification of blood and semen and their characters (Barni et al., 2007).

B- Preliminary tests:-

Each piece of cotton fabric was divided into two equal halves. Presumptive tests: Kastle Mayer test (phenolphthalein test) for blood samples and Florence test for semen samples were done for half of the piece of the cotton fabric.

C-DNA analysis:

The other half of the cotton fabric was used for DNA analysis.

1) DNA extraction from blood and seminal stain ;

First, the blood and seminal stain samples were extracted from half of the piece of the cotton fabric by addition of 600 μg of lysis buffer to the piece of the cotton fabric in Eppendorf tube, incubated at room temperature for one hour, then vortexed for 15 sec. and centrifuged at 13,000 for 1 min. (Hue et al., 2012).

2) Purifying genomic DNA from whole blood and semen:

Blood DNA was extracted using DNA extraction kit (Bioline Company, Cat No. BIO_52063) following the manufacturer protocol (Ahmed et al., 2011). Seminal DNA also was extracted using DNA extraction kit, (Bioline Company, Cat No. BIO_52066) following the manufacturer protocol (Timken et al., 2005)**.**

3) DNA quantification by spectrophotometer for blood and semen samples:

After extraction, quantity of extracted DNA is estimated by NanoDrop Spectrophotometer ND-1000. DNA absorbs UV light in a specific pattern. In a Spectrophotometer cuvette, 5 um of the sample was exposed to UV light at 260 nm and 280 nm, and a photo-detector measured the light that passes through the sample. The concentration of extracted DNA was assessed at 260 nm and 280 nm. The ratio between the reading at 260 and 280 nm (OD 260/280) provided an estimation of purity of DNA (Hue et al., 2012).

4) DNAamplification

This was done by using the PCR, following the kit protocol of PCR Tag, Bioline Company (Schaerli et al., 2008).

PCR reaction set up:

a. Five taq red reaction buffer (10 μl).

b. Template (1 to 2μl).

c. Primers (20 μM each) (0.5 μl).
-TH01 forward 5′-ATTCAAAGGGTATCTGGGCTCTGG-3′. (which referes to intron 1 of the tyrosine hydroxylase gene)
-TH01 reverse5′-GTGGGCTGAAAAGCTCCCGATTAT3′.
-Amelogenin forward5′-ACCTCATCCTGGGCACCCTGGTT3′.
-Amelogenin reverse5′-AGGCTTGAGGCCAACCATCAG3′.

d. Taq HS Red Mix, 2x (12.5μl).

e. Water (dH$_2$O) (up to 25μl).

5) PCR cycling conditions:

PCR was carried out on the extracted DNA samples. The PCR was conducted as the following; denaturation at 95°C for 11 min; 28 cycles of denaturing at 94°C for 1 min. Then, annealing at 59°C for 1 min. Extension at 72°C for 1 min and finally extension at 60°C for 60 min. (Gibson et al., 2010).

6) Analysis of amplification products;

Amplification products of blood and semen DNA were compared to control using gel electrophoresis (Gibson et al., 2010).

Statistical analysis:

Collected data were analyzed using statistical program of social science (SPSS) version 20. The data were expressed as mean

± standard deviation and the difference between means was compared using Student's t-test.

III- RESULTS

All samples, either blood or semen were analyzed to detect the effect of aging process, firstly on the macroscopic appearance of the stains followed by presumptive tests to detect the nature of the specimen (blood or semen), finally quantitative and qualitative analysis of DNA.

1- Effect of aging on bloodstains:

A- Macroscopic appearance of bloodstains after exposure to aging process.

Figure (1) shows the effect of exposure to aging on the macroscopic appearance of the bloodstain on the cotton fabric in comparison to the control. Bloodstains left for one, three and six months became darker in color compared to the control.
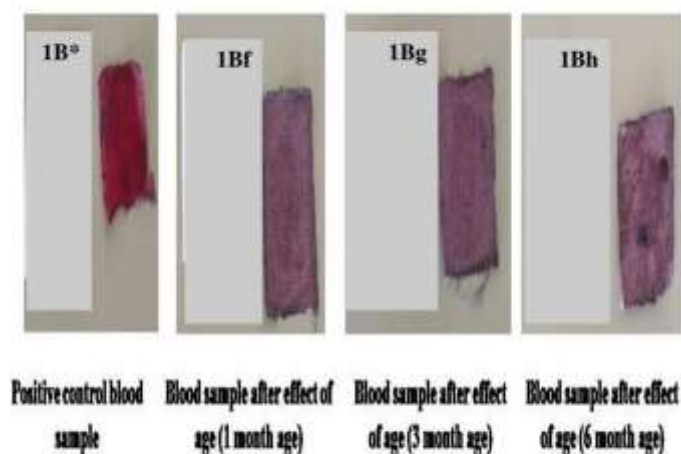

Figure (1): The effect of exposure to aging on the macroscopic appearance of the bloodstain on the cotton fabric in comparison to the control.

B- Presumptive test of bloodstains after exposure to aging process.

Figure (2) represents the effect of exposure to aging on detection of bloodstain on the cotton fabric by phenolphthalein test in comparison to the control. Bloodstains, which left for one, three and six months, gave positive results as strong in color as the control within 2 seconds of the test.
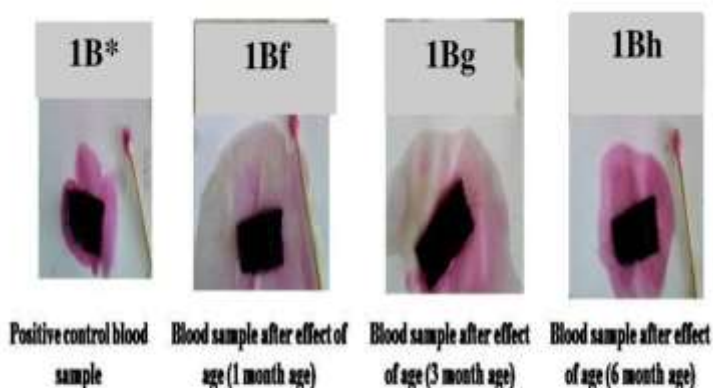

Figure (2): Effect of exposure to aging on detection of bloodstain on the cotton fabric by phenolphthalein test in comparison to the control.

C- DNA concentration in bloodstains after exposure to aging process.

Table (1) shows the effect of exposure to aging on blood DNA concentration (ng/µl) measured by spectrophotometer in comparison to the control. There is highly significant reduction in the concentration of blood DNA extracted from bloodstains left for one, three and six months with mean value ± SD (66.90±11.09, 58.40±12.33, 39.20±3.76) respectively.

Table (1): Effect of exposure to aging on blood DNA concentration (ng/µl) measured by spectrophotometer in comparison to the control by student t- test

(N=20)	Blood DNA conc.(ng/ul)		P- value
	Mean	**±S.D**	
Group 1 (positive Control)	80.92	±3.56	
Group 2 (After 1 month)	66.90	±11.09	<0.001**
Group 3 (After 3 months)	58.40	±12.33	<0.001**
Group 4 (After 6 months)	39.20	±3.76	<0.001**

SD = Standard deviation.
**: P-value ≤ 0.001 highly significant.
N= number of subjects

D- Analysis of DNA primers by gel electrophoresis of bloodstains after exposure to aging process.

Figure (3): represents PCR amplified product of TH01 primer and Amelogenin primer for positive and negative control of bloodstains by using 2% agarose gel electrophoresis stained with EthB. (Lane 1) 50 bp DNA marker. (Lane 2) positive control by TH01 primer. (lane3) positive control by Amelogenin primer.(Lane 4) negative control by TH01. (lane5) negative control by Amelogenin. The bands in the lane 2 are two bands at 200 and 210 bp**.** The bands in lane 3 are three bands at 210, 230 and 60 bp. No bands appear in lane 4, 5.
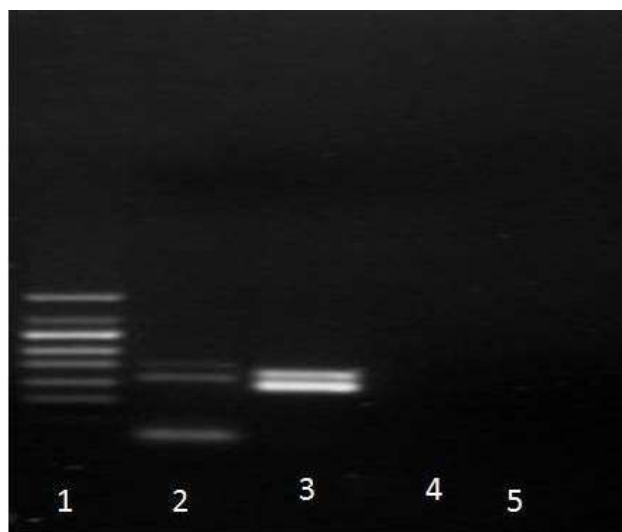

Figure (3): 2% agarose gel electrophoresis stained with EthB showing PCR amplified product of TH01 primer and Amelogeninprimer for positive and negative control of bloodstains.
(lane 1) 50 bp DNA marker.
(lane 2) positive control by   TH01 primer.
(lane 3) positive control by Amelogenin primer.
(lane 4) negative control by TH01.
(lane5) negative control by Amelogenin.

Figure (4) represents PCR amplified product of TH01 primer for the effect of exposure to aging on detection of bloodstain on the cotton fabric in comparison to the control by using 2% agarose gel electrophoresis stained with EthB. (Lane 1) 50 bp DNA marker. (Lane 2) positive control. (lane3) bloodstain left at room temperature for 1 month. (Lane 4) bloodstain left at room temperature for three months. (lane 5) bloodstain left at room temperature for six months. (Lane 6) negative control. The bands in the lane 3, 4 and 5 appear as the control in the form of two bands at 200 and 210 bp. No bands appear in lane 6.
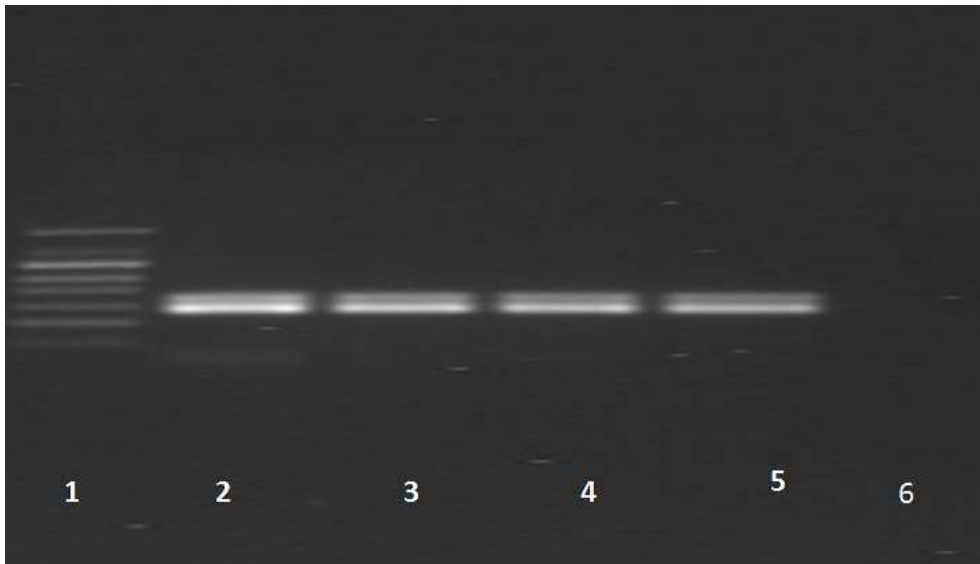

Figure (4):2% agarose gel electrophoresis stained with EthB showing PCR amplified product of TH01 primer for the effect of exposure to aging on detection of bloodstain on the cotton fabric in comparison to the control.

(lane 1) 50 bp DNA marker.
(lane 2) positive control.
(lane3) bloodstain left at room temperature for 1 month.
(lane 4) bloodstain left at room temperature for 3 months.
(lane 5) bloodstain left at room temperature for 6 months.
(lane 6) negative control.

Figure (5) represents PCR amplified product of Amelogenin primer for the effect of exposure to aging on detection of bloodstain on the cotton fabric in comparison to the control by using 2% agarose gel electrophoresis stained with EthB. (Lane 1) 50 bp DNA marker.(Lane 2) positive control. (lane3) bloodstain left at room temperature for 1 month. (Lane 4) bloodstain left at room temperature for three months. (Lane 5) bloodstain left at room temperature for six months. (Lane 6) negative control. The bands in the lane 3 and 4 appear as the control in the form of three bands at 210, 230 and 60 bp. No band appears in lane 5 and 6.
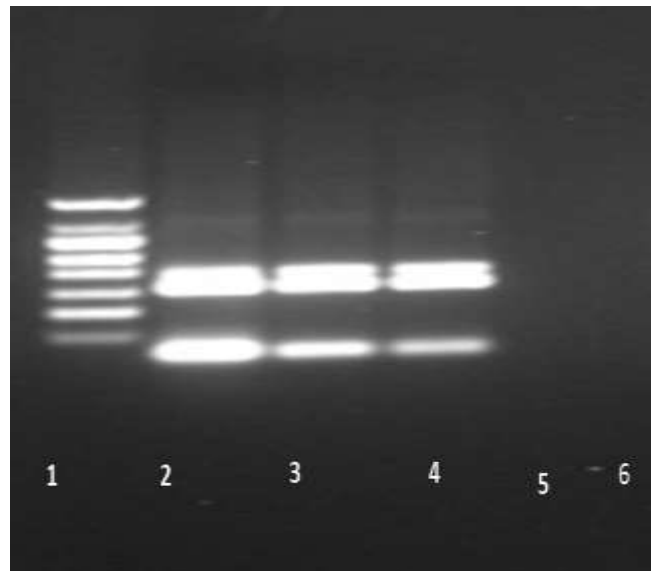
Figure (5) : 2% agarose gel electrophoresis stained with EthB showing PCR amplified product of Amelogenin primer for the effect of exposure to aging on detection of bloodstain on the cotton fabric in comparison to the control.

(lane 1) 50 bp DNA marker.
(lane 2) positive control.
(lane3) bloodstain left at room temperature for 1 month.
(lane 4) bloodstain left at room temperature for 3 months**.**
(lane 5) bloodstain left at room temperature for 6 months**.**
(lane 6) negative control.

2- Effect of aging process on seminal stains:
A- Macroscopic appearance of seminal stains after exposure to aging process.

    Figure (6) shows the effect of exposure to aging on the macroscopic appearance of the seminal stain on the cotton fabric. The colour of seminal stains left for one, three and six months became deep yellow compared to the control.
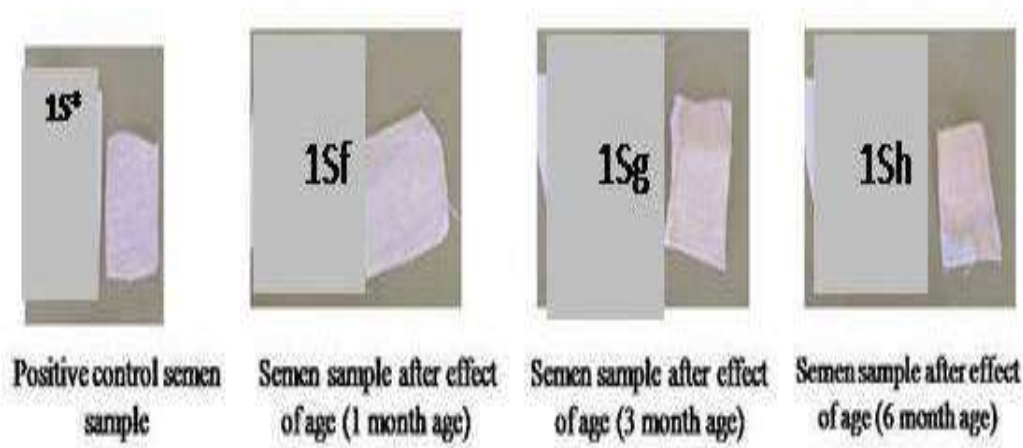

Figure (6) Effect of exposure to aging on the macroscopic appearance of the seminal stain on the cotton fabric in comparison to the control.

B- Presumptive test of seminal stains after exposure to aging process.
    Figure (7) shows the effect of aging on detection the seminal stains on the cotton fabric by Florence test in comparison to the control. Seminal stains left for one, three and six months gave

positive results in the form of brown rhombic shaped crystals with Florence reagent examined by light microscope, magnification power is 10X.
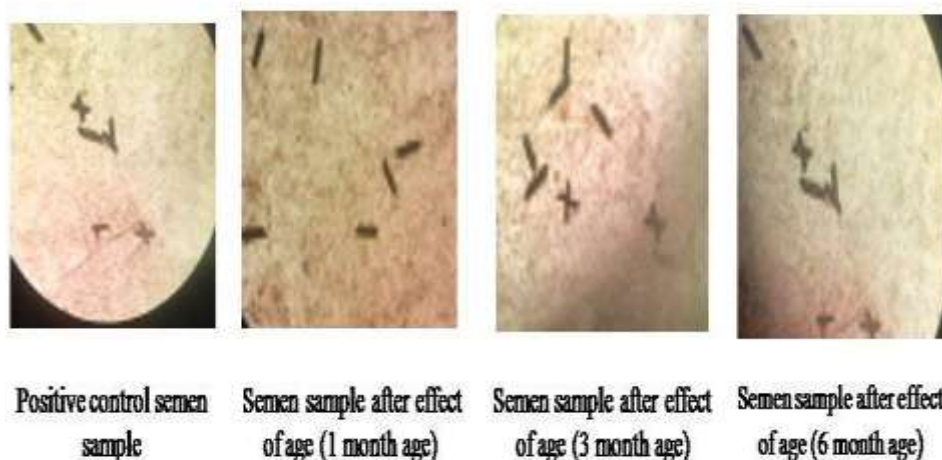

Positive control semen sample   Semen sample after effect of age (1 month age)   Semen sample after effect of age (3 month age)   Semen sample after effect of age (6 month age)

Figure (7): Effect of aging on the detection of seminal stain on the cotton fabric by Florence test in comparison to the control.

C- DNA concentration of semen stains after exposure to aging process.

Table **(2)** represents the effect of exposure to aging on semen DNA concentration (ng/µl) measured by spectrophotometer in comparison to the control. There is significant reduction in the concentration of semen DNA extracted from seminal stains left for one month, three months and six months before processing with mean value± SD (68.25±7.70, 53.90±7.33, 45.75±8.17) respectively compared to the control.

Table (2): Effect of exposure to aging on semen DNA concentration (ng/µl) measured by spectrophotometer in comparison to the control by student t-test.

N=20	Semen DNA conc.(ng/ul)		
	Mean	**±S.D**	**P- value**
Group 1 (positive Control)	90.90	±2.845	
Group 2 (After 1 month)	68.25	±7.70	<0.001***
Group 3 (After 3 months)	53.90	±7.33	<0.001***
Group 4 (After 6 months)	45.75	±8.17	<0.001***

SD = Standard deviation.
*P-value ≤ 0.05 significant.
**: P-value ≤ 0.001 highly significant.
N= number of subjects

D-Analysis of DNA primers by gel electrophoresis of seminal stains after exposure to aging process.

Figure **(8)** represents PCR amplified product of TH01 primer and Amelogenin primer for positive and negative control of seminal stains. (lane 1) 50 bp DNA marker by using 2% agarose gel electrophoresis stained with EthB. (Lane 2) positive control by TH01 primer. (lane3) positive control by Amelogenin primer. (Lane 4) negative control by TH01. (lane5) negative control by Amelogenin. The bands in the lane 2 are two bands at 200 and 210 bp**.** The bands in lane 3 are three bands at 210, 230 and 60 bp. No bands appear in lane 4, 5.
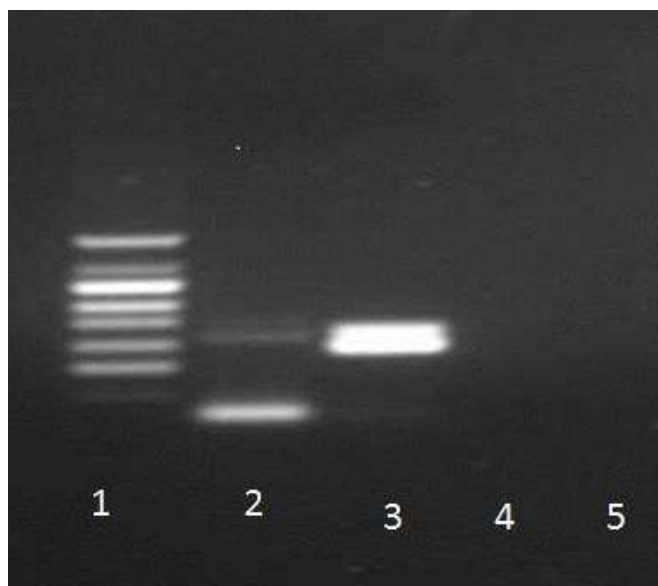
Figure (8): 2% agarose gel electrophoresis stained with EthB showing PCR amplified product of TH01 primer and Amelogeninprimer for positive and negative control of semen stains.

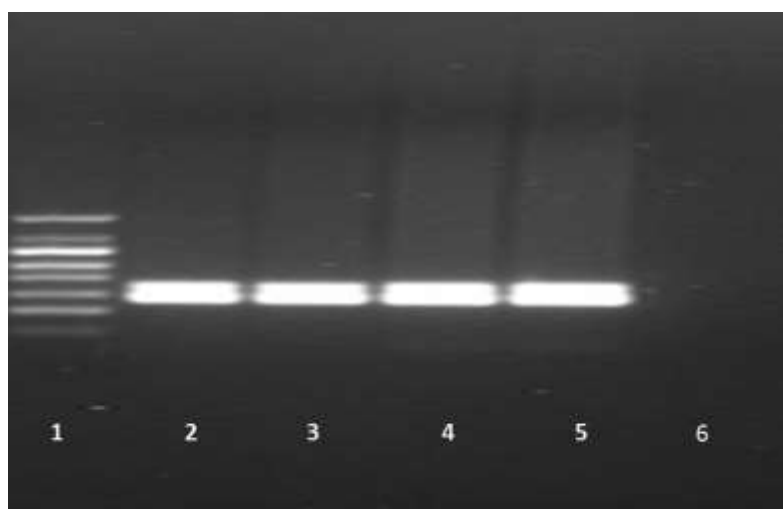(lane 1) 50 bp DNA marker.
(lane 2) positive control by TH01 primer .
(lane3) positive control by Amelogenin primer.
(lane 4) negative control by TH01 .
(lane5) negative control by Amelogenin.
Figure (9) represents PCR amplified product of TH01 primer for the effect of exposure to aging on detection of seminal stain on the cotton fabric in comparison to the control by using 2% agarose gel electrophoresis stained with EthB. (lane 1) 50 bp DNA marker. (lane 2) positive control. (lane3) seminal stain left at room temperature for 1 month. (Lane 4) seminal stain left at room temperature for 3 months. **(**lane 5) seminal stain left at room temperature for 6 months**.** (lane 6) negative control. The bands in the lane 3, 4 and 5 appear as the control in the form of two bands at 200 and 210 bp. No bands appear in lane 6.

Figure(9): 2% agarose gel electrophoresis stained with EthB showing PCR amplified product of TH01 primer for the effect of exposure to aging on detection of seminal stain on the cotton fabric in comparison to the control. (lane 1) 50 bp DNA marker.

(lane 2) positive control.

(lane3) seminal stain left at room temperature for 1 month.

(lane 4) seminal stain left at room temperature for 3 months.

(lane 5) seminal stain left at room temperature for 6 months.

(lane 6) negative control.

Figure (10) represents PCR amplified product of Amelogenin primer for the effect of exposure to aging on detection of seminal stain on the cotton fabric in comparison to the control by using 2% agarose gel electrophoresis stained with EthB. (lane 1) 50 bp DNA marker. (lane 2) positive control.(lane3) seminal stain left at room temperature for 1 month. (lane 4) seminal stain left at room temperature for 3 months.(lane 5) seminal stain left at room temperature for 6 months.(lane 6) negative control. The bands in the lane 3, 4 and 5 appear as the control in the form of three bands at 210 , 230 and 60 bp. No bands appear in lane 6.
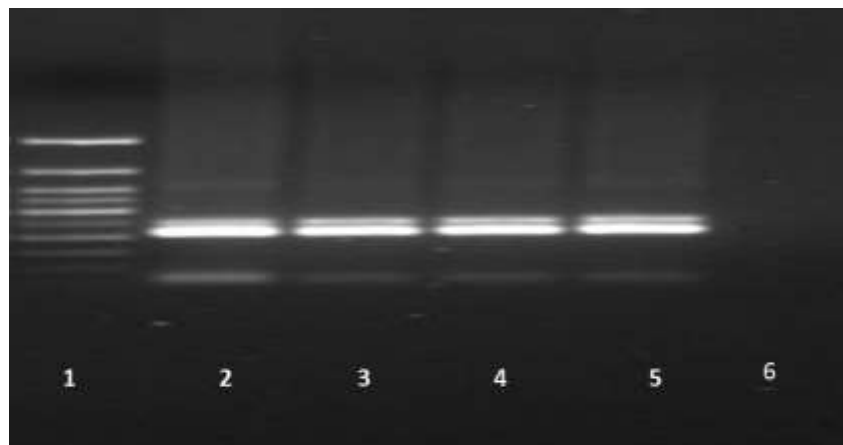

Figure (10): 2% agarose gel electrophoresis stained with EthB showing PCR amplified product of Amelogenin primer for the effect of exposure to aging on detection of seminal stain on the cotton fabric in comparison to the control.

(lane 1) 50 bp DNA marker.

(lane 2) positive control.

(lane3) seminal stain left at room temperature for 1 month.

(lane 4) seminal stain left at room temperature for 3 months.

(lane 5) seminal stain left at room temperature for 6 months.

(lane 6) negative control .

3-   Comparison between the stability of bloodstain samples and seminal stain samples after exposure to aging process:

A-   Presumptive tests of blood and semen stains after exposure to aging process.

Table (3): shows comparison between the effect of aging on detection of blood and semen stains by presumptive tests (phenolphthalein for bloodstain and Florence test for seminal stain).Blood and semen stains which left for one month , three months and six months gave positive results**.**

Table (3): Comparison between the effect of aging on detection of blood and seminal stains by presumptive tests (phenolphthalein for bloodstain and Florence test for semen stain).

	Positive control (N=20)	After 1 mo. (N=20)	After 3 mo. (N=20)	After 6 mo. (N=20)
Phenolphthalein test	+ve	+ve	+ve	+ve
Florence test	+ve	+ve	+ve	+ve

N= number of subjects.

B- DNA concentration:

Figure (11) represents comparison between level of DNA concentration percentage in blood and seminal stains after exposure to aging process. There was significant difference in the percentage of concentration of DNA between blood and seminal stains left for three months with higher DNA concentration obtained from blood samples. There was no significant difference in the concentration of DNA between blood and seminal samples left for one month and six months.
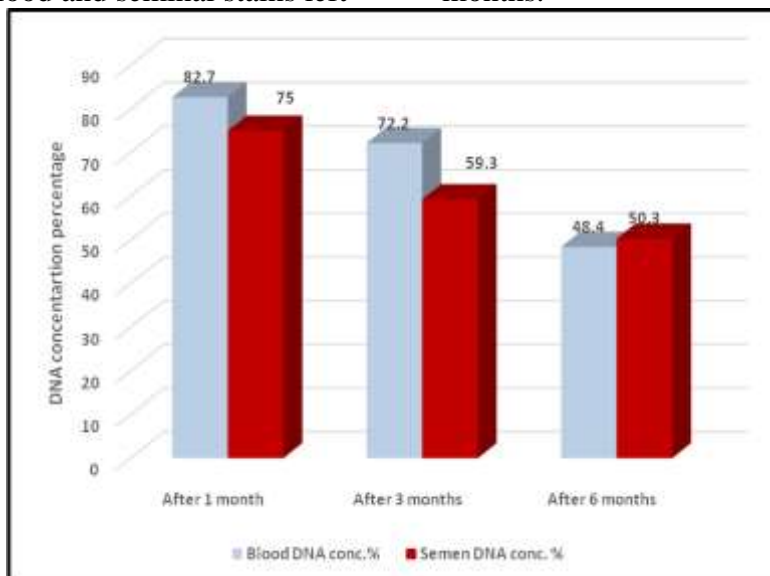

Figure (11): Comparison between level of DNA concentration percentage in blood and seminal stains after exposure to aging process.

C- Analysis of D NA primers by gel electrophoresis.

Table (4) represents comparison between blood and semen samples after exposure to aging by TH01 and Amelogenin analysis. Both blood and seminal stains can be detected by using TH01 primer. Also, blood and seminal stains can be detected by using Amelogenin primer for samples left for one month and three months before processing. Bloodstains left for six months cannot be detected by Amelogenin primer while, seminal stain left for six months can be detected by Amelogenin primer.

Table (4): Descriptive comparison between blood and seminal stains after exposure to aging by TH01 and Amelogenin analysis.

		Positive control (n=20)	After 1 mo. (N=20)	After 3 mo. (N=20)	After 6 mo. (N=20)
TH01	**Blood**	+ve	+ve	+ve	+ve
	Semen	+ve	+ve	+ve	+ve
Amelogenin	**Blood**	+ve	+ve	+ve	-ve
	Semen	+ve	+ve	+ve	+ve

N= number of subjects.

IV- DISCUSSION

A forensic laboratory and pathologist, usually deal with low quality samples. These samples in the form of liquid blood sample or more often as a bloodstain which sometimes contain inhibitant to the DNA analysis or PCR amplification (Barbaro and Cormaci, 2006)**.**

The exposure to different period of storage may affect the bloodstain at the level of macroscopic appearance, identification by preliminary tests and DNA quality and

quantity. In the current research, aging of the bloodstains affected their macroscopic appearance. Bloodstains left for one, three and six months became darker in color because it reacted with oxygen, and the majority of the water in blood evaporated, making a more concentrated pigmentation. This result was in agreement with Fabbri et al. (2017) in their study about the examination of blood traces on different fabrics. They found that old dry bloodstains had a dark colour than the control stains.

Presumptive test (phenolphthalein) was performed in the present study to identify blood in bloodstains after exposure to aging process and the results showed that, blood can be detected by phenolphthalein test in bloodstains left for one, three and six months before processing. This is due to stability of the oxidase enzyme over time which is responsible for the positive results of phenolphthalein test. This finding is also mentioned by Turrina et al. (2008) in their study about identification of old bloodstains by presumptive tests including phenolphthalein test. They stated that blood could be identified by phenolphthalein test in bloodstains that have been stored at room temperature.

As time increased, DNA began to degrade gradually. In the current study, DNA concentration was significantly reduced in bloodstains left for one, three and six months before processing. As the extract ability of DNA from whole blood depends upon the viability of the leucocytes, which decrease by time. This result was in agreement with Huang et al. (2017) in their study about the effects of storage time on the DNA quantity and quality. They stated that there is dramatic decrease in DNA concentration after 15 days storage.

Quality of DNA was not affected by storage length in the present research (one, three and six months). DNA fragments of PCR amplified products of TH01 primer could be identified by using gel electrophoresis. This attributed to the fact that DNA in bloodstains is most likely still entrapped in the nucleus and tightly bound to histones. This might offer some protection against degradation as

ascertained by Dissing et al. (2010) who identified DNA after seven months storage at room temperature (35˚C). On the other side by using Amelogenin primer, DNA fragments could be identified by using gel electrophoresis of PCR amplified products for bloodstains left for one and three months but cannot detect DNA in bloodstains left for six months before processing as reported byRahikainen et al. (2016)in their study about DNA quality and quantity in post-mortem bloodstains. They stated that the shorter fragments amplify better than longer fragments in samples of old age.

Seminal stains left at a crime scene may become the first clue of the crime. It can discriminate the suspect by DNA analysis and typing. (Nakanishi et al., 2014).

Macroscopic image of the seminal stains after exposure to aging process was demonstrated in the current research. The color of seminal stains left for one, three and six months became deep yellow compared to the control.

Regarding the presumptive test (Florence test) which was performed to identify seminal stains after exposure to aging process, The current study showed that choline periodide crystals (brown rhombic shaped crystals) could be detected with Florence test for seminal stains left for one, three or six examined by light microscope, magnification power is 10X.

In the present study, DNA concentration was significantly reduced in seminal stains left for one, three and six months before processing. This is attributed to degradation of DNA as the time of storage increases. This result was in agreement with Bini et al. (2015) who reported in their study about the degradation of DNA in old seminal stains that the number of detected loci is decreased as the storage duration increase due to DNA degradation.

Quality of DNA was not affected by storage length in the current study (one, three and six months). DNA fragments of PCR amplified products of TH01 and Amelogenin primer could be identified by using gel electrophoresis. This is attributed to the fact that sperm chromatin is tightly compacted which leads to its stability. As ascertained by Bini et al. (2015) who confirmed that STR

analysis could be used for extremely aged seminal stain samples and may be useful in forensic practice and criminal trials.

Regarding, the stability of the blood and seminal stains, there was no significant difference between identification of blood and seminal stains by presumptive tests when they left for one, three, and six months before processing and both types of samples could be identified.

In the present study, there were no significant differences between the percentage of DNA which could be extracted from bloodstains and seminal stains left for one month and six months but higher percentage of DNA in bloodstains left for three months was detected compared to the percentage of DNA in seminal stains left for the same period. This is probable due to the difference in the ambient room temperature between samples left for three months (March, April and May) and samples left for six months (from March to August) (Ali, 2012).

There was no significant difference between the quality of DNA extracted from blood and seminal stains left for one, three and six months before processing except for bloodstains left for six months before processing that cannot be detected by Amelogenin primer while seminal stains can be detected by Amelogenin primer. So, semen DNA is more resistant to aging process than blood DNA by using Amelogenin primer.

Comparing between the two primers used in the present study (TH01 and Amelogenin), the results showed that TH01 is more resistant to the effect of aging especially in seminal stains and it is better than Amelogenin which gave negative results with seminal stains left for six months before processing.

V- CONCLUSION

Storage duration of blood and seminal stains did not affect the ability to identify them by preliminary tests. Storage duration caused reduction in DNA concentration but did not affect the ability to obtain DNA except for bloodstains left for six months by using Amelogenin primer but can be detected by using TH01 primer. Standard procedures should be used with crime scene samples to minimize the loss of important evidence, which can be the only clue for the crime.

VI- RECOMMENDATION

1- It is recommended to expose the samples to longer period more than 6 months at room temperature.

2- Different primers can be used for further research.

3- Other types of samples can be used in further studies rather than blood and semen as saliva and teeth.

VII- Funding

VIII- Conflict of interest
None

IX- Acknowledgment

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دور ترك العينات لفترات زمنيه مختلفه في تحديدالحمض النووي المستخرج من البقع الدمويه والبقع المنوية

رنده حسين عبد الهادى ،هيام زكريا ثابت ،هبه عطيه يسى ،نها اسماعيل ابراهيم

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يلعب فحص الحمض النووي دورًا حيويًا أكثر من أي وقت مضى في القضايا الجنائية ، حيث أن العينات التي يتم استخلاصها من مسرح الجريمة قد تتعرض لظروف مختلفة قبل المعالجه مما قد يؤدي إلى تدهور الحمض النووي. ومن هذه الحالات تأثير ترك العينه لفترات زمنيه مختلفه . **الهدف من هذه الدراسة** هو تقييم تأثير ترك العينه لفترات زمنيه مختلفه على استخراج الحمض النووي من البقع الدمويه والبقع المنوية. وقد أجريت هذه الدراسة على عشرين متطوعًا ذكرا؛ تم جمع أربعة مل من الدم واثنين مل من السائل المنوي من نفس المتطوع وتم وضعها على قطع من نسيج القطن. تم اختبار تأثير ترك العينات لمده (شهر واحد وثلاثة وستة أشهر) على امكانيه استخراج الحمض النووى من الدم والسائل المنوى. أولاً، تم تحليل العينات عن طريق اختبارات أولية ثم تم استخراج الحمض النووي من البقع الدمويه والمنويه، و قياس تركيز الحمض النووي بواسطة مقياس الطيف الضوئي وتحليلها باستخدام الفصل الكهربائي الهلامى النتائج قد تبين أن البقع الدمويه والبقع المنوية التى تركت في درجة حرارة الغرفة لمدة شهر واحد وثلاثة وستة أشهر امكن التعرف عليها باستخدام اختبارات اوليه. وقل تركيز الحمض النووي بشكل كبير في البقع الدمويه والمنويه التي تركت لمدة شهر واحد وثلاثة وستة أشهر قبل المعالجة. لم تتأثر جودة الحمض النووي بطول التخزين وامكن تحديد جزيئات الحمض النووي باستثناء بقع الدم التي تركت لمدة ستة أشهر والتي لم يتمكن الكشف عنها بواسطة أميلوجينين. **الخلاصه:** امكن استخراج الحمض النووى من البقع الدمويه والمنويه التى تركت لمده شهر وتلاته اشهر وسته اشهر فى درجه حراره الغرفه يوصى بتعريض العينات لفترات أطول من 6 أشهر عند درجة حرارة الغرفة. يمكن استخدام بادئات مختلفة فى ابحاث اخرى و يمكن استخدام أنواع مختلفه من العينات في دراسات أخرى بدلاً من عينات الدم و السائل المنوى مثل اللعاب والأسنان.