EFFECT OF DIFFERENT HUMAN MUSCLE TISSUE PRESERVATIVES ON QUALITY AND QUANTITY OF DNA: MEDICO LEGAL ASPECT

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ABSTRACT

Background: Disaster victim identification (DVI) is a part of a collective response to mass disasters with the intention of identifying victims and body parts. Muscle tissue is sampled during DVI operations to identify victims by DNA analysis. **Aim:** To provide an effective field based method for preservation of DNA in human muscle tissues. **Methods:** A cross sectional comparative study was carried out on normal healthy human muscle tissues which were collected from patients admitted to Suez Canal University Hospital for surgical amputation of a part of limb. Muscle tissue was stored for 4 weeks in a number of preservatives at room temperature (15-20°C). This process was repeated at 37°C to simulate the conditions expected during DVI operations in warm conditions. Samples were also stored at -20ºC which represents the optimum storage condition used in many countries. Quantitative and qualitative analysis of DNA extracted from samples was done for each preservative during the 4 weeks. The study was conducted in the clinical pathology department in faculty of medicine-Suez Canal University, Ismailia, Egypt. **Results:** All preservatives could retain DNA up to the 4 weeks. Ethanol 70% (EtOH) gave the highest DNA concentration in both conditions (**p** value <0.001), followed by Dimethyl sulfoxide (DMSO) in room temperature and Sodium Chloride (NaCl) in 37 ºC. All used preservatives did not show degradation in room temperature, while NaCl and Ethanol 70% showed degradation in DNA extracted in day 28 in 37 ºC condition (**p** value <0.001). **Conclusion:** DMSO is a successful method of preservation of DNA in human muscle tissue up to 4 weeks in both room temperature and 37 ºC. While NaCl and Ethanol 70% are successful methods of preservation of DNA in muscle tissue up to 2 weeks only in 37 ºC and up to 4 weeks in room temperature (15-20ºC).

Key words: Disaster victim identification; Forensic DNA analysis; preservatives; Ethanol, DMSO.

INTRODUCTION

Identification is an important task in Forensic Medicine whether it involves an individual crises or a mass disaster. In Mass disasters, this task is very challenging and the ability to recognize victims is very critical as the extent of a disaster may hold back and delay identification **(Interpol, 2009).** Moreover, Forensic and medical staff members usually face lack of resources, as in 2004, Asian tsunami **(Bajaj, 2005; Morgan et al., 2006; Tsokos et al., 2006).**

DNA examination is one of the primary three identification methods recommended by the International Criminal Police Organization (INTERPOL) **(INTERPOL, 2009; Sweet, 2010).** As soft tissue is usually abundant in mass disasters, deep red non-decomposing muscles are recommended for DNA profiling when available. However, the main problem facing the forensic staff is to get the samples to the lab in proper conditions **(Prinz et al., 2007)** as by the time they reach the labs, they are either partially or completely degraded and DNA can't be extracted from them **(Dassauer et al., 1996).** Therefore, rapid collection and storage of samples through optimizing the conditions responsible for DNA degradation, e.g. temperature, duration and preservatives are mandatory for successful DNA profiling **(Dassauer et al., 1996**; **Prinz et al., 2007)**.

A lot of published research reported methods to preserve tissues for medium to long periods of time **(Kilpatrick, 2002; Caputo et al., 2011)**. However, mass disasters are generally field-based that requires short-term storage because the samples will be stored in refrigerators following transportation to the laboratory **(Allen-Hall and McNevin, 2012).**

Known procedures for maintaining biological samples vary widely between placing in cold storage as recommended by **The National Association of Medical Examiners, (2005)** and **Federal Bureau of Investigation, (2007)** and preserving briefly in preservative solutions in room temperature **(Fregeau et al., 2001).** All of these methods have advantages and disadvantages. Cold storage is widely used, as freezing reduces DNA changes, rendering DNA extractions in no need for any special considerations. However, this method requires refrigerators, a power supply or available liquid nitrogen. On the other hand, dehydration prevents nuclease and bacteria by removal of water **(Gillespie et al., 2002).**

Storage in a liquid preservative has been the focus of various studies. Alcohol is a common preservative solution which is effective at dehydrating and sterilizing samples without cross-linking DNA. On the contrary, formaldehyde which permeates tissue and cross links proteins and nucleic acids affects the quality of DNA extraction **(Gillespie et al., 2002; Srinivasan et al., 2002).** Ethanol is an inexpensive and easily obtained alcohol. It has proven to be an effective tissue storage method that allows DNA recovery **(Dawson et al., 1998; Penna et al., 2001; Gillespie et al., 2002; Kilpatrick, 2002)** as well as killing a wide range of bacteria and fungi **(Fregeau et al., 2001)**. Other less common tissue storage solution is dimethyl sulfoxide (DMSO) which is highly permeable in tissues, dehydrating them by displacing water. However, when it is combined with

Sodium Chloride (NaCl) and EDTA, DMSO has been proved to be very effective at preserving DNA **(Kilpatrick, 2002).**

Most of the preservatives used are liquid based; however, solid based preservatives are very effective in field as there is a very little chance of spillage or evaporation over time. Sodium chloride (NaCl) is a common preservative that has been used for centuries. In solid form, it dehydrates the sample, inactivates nucleases and slows the growth of bacteria **(Nagy, 2010).** The present study was carried out to compare between the effect of different field based tissue preservatives at both room temperature (15-20 ºC) and 37 ºC on the quantity and quality of DNA recovery for further identification in an attempt to provide an effective field based method for preservation of DNA in human muscle tissues

MATERIALS & METHODS

A cross sectional comparative study was carried out on normal healthy human muscle tissues which were collected from two patients admitted to Suez Canal University Hospital for surgical amputation of a part of a limb. The study protocol runs in compliance with the Helsinki Declaration and approved by Research Ethics Committee of Faculty of Medicine Suez Canal University. An informed written consent was obtained from the two participants. All data were confidential as anonymized residual samples were completely unknown to the researcher. All samples used in this study would not be used in the future in further studies. The study was carried out on two phases. First experiment: tissues which were collected from the first patient, were placed in clean plastic bags, preserved in ice, and then transported to the clinical pathology department laboratory. They were placed in -20 ºC till the next day. On next morning, they were dissected into 91 tissue specimens on a sterilized surface inside a laminar flow cabinet and weighed into 300 mg slices. The tissue slices (21 samples for each preservative) were placed in sterile screw top test tubes in 2 mL of each of the following liquid preservatives: Ethanol 70% and DMSO. Regarding NaCl, tissue slices were placed in 4 g NaCl. Twenty-one tissue slices were put in empty test tubes and preserved at -20 ºC. Seven samples were used without any preservatives for DNA extraction at day 0 to record DNA baseline concentration (zero level). This work was done in winter (December 2013 and January 2014); room temperature was about 15-20 ºC. In the second experiment, the work was repeated using the second patient's tissue sample. Preserved samples were incubated at 37ºC. Muscle tissues embedded into different preservatives were subjected to DNA extraction at days 7, 14 and 28. Each sample was removed from preservative and put in a new tube with one ml distilled water. The sample was centrifuged at maximum speed (12.000 rpm) for 1min. Then distilled water was removed from the tube.

DNA extraction from muscle: DNA was extracted by using Invisorb® spin tissue mini kit for isolation of total genomic DNA from tissue. Extraction was undertaken following manufacturer recommendations. After extraction, DNA concentration of each sample was measured by using the NanoDrop ® ND-1000, Full-spectrum UV/Vis

Spectrophotometer (NanoDrop Technologies, Inc. Wilmington, DE USA, 2005). A rough estimate of DNA quality was made by electrophoresing the samples on a 0.7% agarose gel. Then gel was carefully exposed to UV trans-illuminator. The bands were analyzed and picture was taken. All equipment used to handle tissue samples were either autoclaved or sterilized using 10% bleach or 70% ethanol.

Data analysis: All statistical analyses were done using SPSS (Statistical Package for Social Sciences) version22. Numeric data were presented in the form of means ± standard deviations. Qualitative data were presented as numbers and percentages. Significance of differences in DNA concentration between the four studied groups (preservation methods) was assessed using Kruskal-Wallis Test. Differences between pair of groups was assessed using Mann-Whitney U test. Wilcoxon Signed Ranks test was used for assessment of significance of difference between two repeated measurements of the same group. Freidman test was used to assess significance of differences in DNA concentration of >2 repeated measurements of the same group. Chi square test was used to assess differences between groups in qualitative data (DNA degradation). Exact p value of Chi-square test was used in case expected count of ≥25% of cells was <5. For all statistical tests level of significance was <0.05.

RESULTS

DNA extracts of excised muscle tissue samples at day 0 (baseline DNA concentration) in the first experiment ranged from 99-101 ng/ mL with a mean value 100.14±0.690 ng/ml, while they ranged from 57-58 ng/ml with a mean value 57.57±0.535 ng/ml in the second experiment. The DNA masses recovered at day 0 and those recovered at days 7, 14 and 28 for each preservation method (first experiment) are shown in table (1), while those recovered after repetition of the experiment at 37 ºC (second experiment) are shown in table (2).

The present study revealed significant differences among the used preservation methods (in both experiments) in DNA concentrations extracted at days 7, 14 and 28. EtOH 70% gave the highest yield followed by DMSO in the first experiment (Kruskal-Wallis Test, p value <0.001). In the second experiment, EtOH 70% gave the highest yield through the whole duration of the study, followed by DMSO in day 7, while it was followed by -20ºC for day 14 and NaCl for day 28 (Kruskal-Wallis Test, p value <0.001). A statistical significant difference was found between each 2 preservatives used in the study for both experiments (Mann-Whitney U test p< 0.05) except for -20°C compared to DMSO at day 28 in the first experiment (at room temperature).

At the first experiment, DNA concentrations extracted from samples preserved in -20ºC, EtOH 70%, DMSO and NaCl, declined over time and declined from baseline DNA except for EtOH 70% which increased over baseline (Friedman test p <0.001) table (1). At the second experiment (incubated at 37 ºC), DNA concentrations extracted from samples which were preserved in Ethanol 70% and NaCl increased overtime, while there was no constant trend for DNA which was extracted in case of DMSO

& -20ºC. They all declined from base line concentrations (Friedman test p <0.001), table (2).

No degradation in DNA extracted from all preserved samples was found over the time periods of the study (up to 4 weeks) in the first experiment figure (1), while at the second experiment, incomplete degradation was found in DNA extracted from 6 out of 7 samples preserved in NaCl and complete degradation of DNA extracted from all samples preserved in Ethanol 70% at day 28 (Exact p value of chi square test <0.001). Otherwise no degradation was found in samples preserved in DMSO & -20ºC figure (2).

Table (1): Concentration of DNA extracted from human muscle tissue along the 28 days of preservation in -20ºC and studied preservatives (NaCl, DMSO, Ethanol 70%) at room temperature (15-20 ºC).

	Storage duration			P value
Preservative	**Day 7***	**Day 14***	**Day 28***	
-20°C	74.9±0.8$^{@\#\$¥}$	20.2±0.9$^{\#\$¥}$	27.7±0.6$^{\#¥}$	<0.001**
NaCl	54.3±0.8$^{@\$¥}$	25.3±0.8$^{\$¥}$	20.3±0.7$^{\$¥}$	<0.001**
DMSO	79.6±0.8$^{@¥}$	42.3±0.5$^{¥}$	28.2±0.7$^{¥}$	<0.001**
Ethanol 70%	117.2±0.7$^{@}$	56.1±0.7	33.1±0.7	<0.001**

All values are presented in mean ± standard deviation
$^{@}$ P value <0.05 compared with baseline
*** P value <0.001 (Kruskal-Wallis Test)**
**** Statistically significant at p <0.05 (Friedman test). Baseline DNA concentration was included (100.1±0.7 ng/ml) for all groups**
$^{\#}$ P value <0.05 compared with NACL group
$^{\$}$ P value<0.05 compared with DMSO group
$^{¥}$ P value<0.05 compared with Ethanol group

Table (2): Concentration of DNA extracted from human muscle tissue along the 28 days of preservation in -20°C and studied preservatives (NaCl, DMSO, Ethanol 70%) at 37 ºC

	Storage duration			
Preservative	**Day 7***	**Day 14***	**Day 28***	**P value**
-20°C	41.4±0.9$^{@\#\$¥}$	64.4±0.6$^{\#\$¥}$	56.1±0.6$^{\#\$¥}$	<0.001**
NaCl	39.4±0.6$^{@\$¥}$	40.3±0.6$^{\$¥}$	114.3±0.5$^{\$¥}$	<0.001**
DMSO	54.2±0.6$^{@¥}$	50.6±0.5$^{¥}$	76.2±0.5$^{¥}$	<0.001**
Ethanol 70%	55.2±0.7$^{@}$	103.9±0.5	115.3±0.7	<0.001**

All values are presented in mean ± standard deviation
$^{@}$ P value <0.05 compared with baseline
*** p<0.001 (Kruskal-Wallis Test)**
**** Statistically significant at p <0.05 (Friedman test). Baseline DNA concentration was included (57.6±0.5 ng/ml) for all groups.**
$^{\#}$ P value <0.05 compared with NACL group
$^{\$}$ P value<0.05 compared with DMSO group
$^{¥}$ P value<0.05 compared with Ethanol group

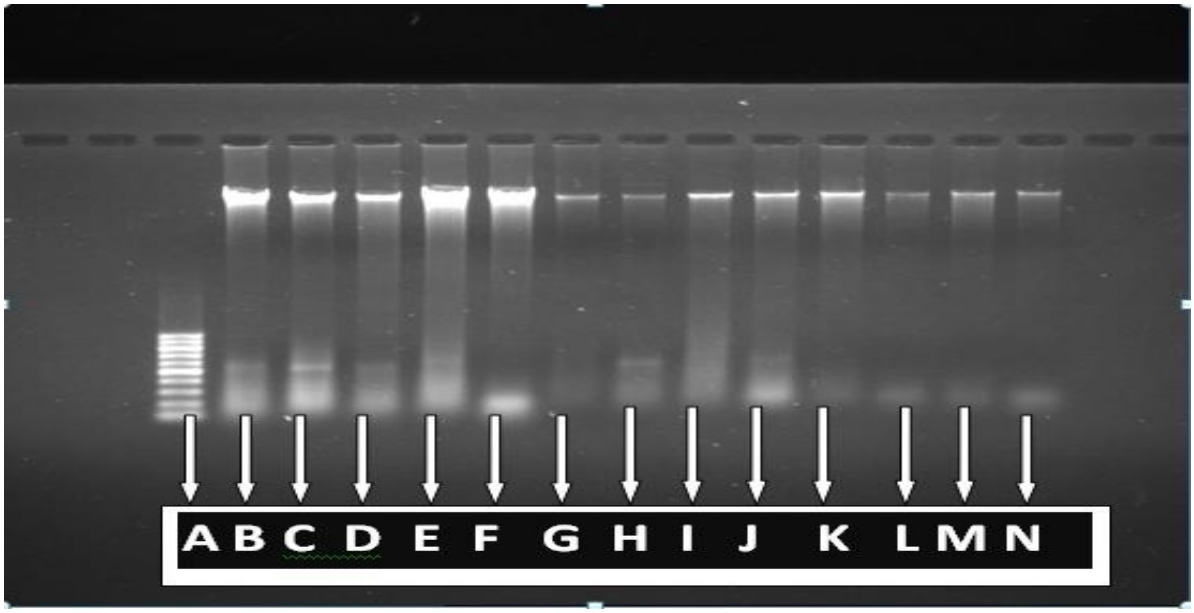

Figure (1) Gel electrophoresis of DNA extracted at days 7, 14 and 28 from human muscle tissues preserved in -20ºC and studied preservatives (NaCl, DMSO, Ethanol 70%) at room temperature (15-20ºC**).**

Samples arranged from left to right as following: A=ladder, B= control, C= -20 ºC (day 7), D= NaCl (day 7), E= DMSO (day 7), F= Ethanol 70 %(day 7), G= -20 ºC (day 14), H= NaCl (day 14), I= DMSO (day 14), J= Ethanol 70 %(day 14),K= -20 ºC (day 28), L= NaCl (day 28), M= DMSO (day 28), N= Ethanol 70 %(day 28).

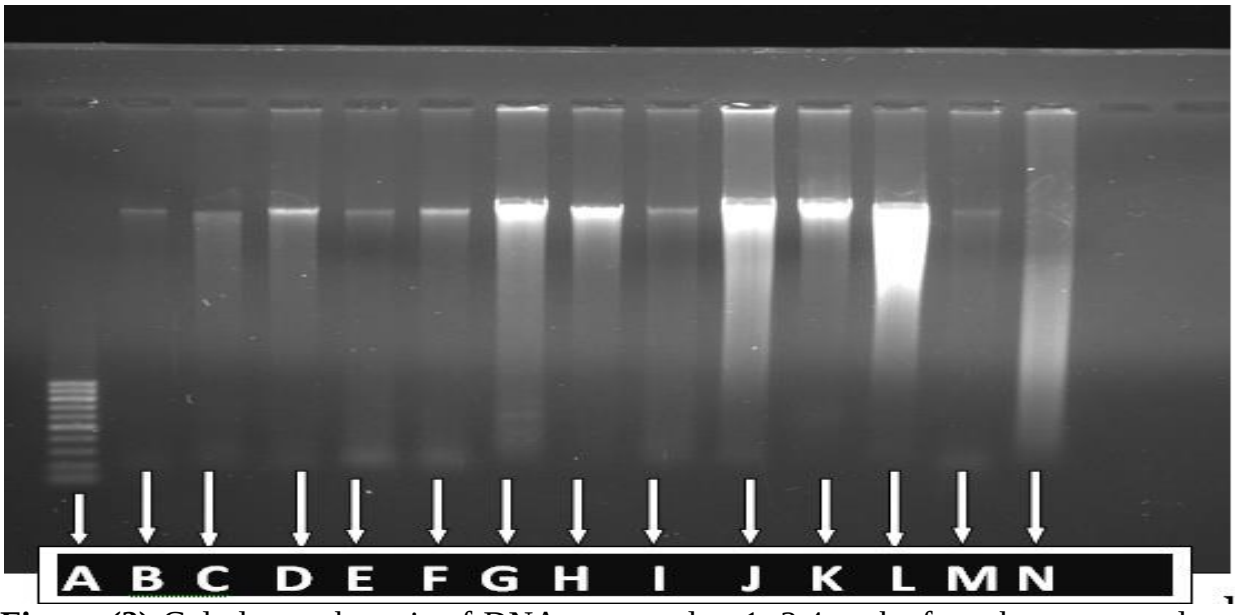

Figure (2) Gel electrophoresis of DNA extracted at 1, 2,4weeks from human muscle tissues preserved in -20ºC and studied preservatives (NaCl, DMSO, Ethanol 70%) at 37ºC.

Samples arranged from left to right as following :A=ladder, B= control ,C= -20 ºC (day 7), D= NaCl (day 7), E= DMSO (day 7), F= Ethanol 70% (day 7), G= -20 ºC (2week), H= NaCl (2week), I= DMSO (2week), J= Ethanol 70 %(2week),K= -20 ºC (day 28), L= NaCl(day 28), M= DMSO(day 28), N= Ethanol 70%(day 28).

DISCUSSION

Disaster victim identification is very challenging due to large number of victims and the presence of fragmented remains. The situation gets more complicated when forensic and medical staff face the lack of facilities, hence emerges the need for preserving tissue samples. Tissue preservation methods should be safe, readily available and easy to transport to the scene with a low cost **(Allen-Hall, 2011)**.

The used preservatives in the present study required little preparation, and did not need any specialized equipment. Moreover, they are inexpensive; therefore they are applicable in developing countries with low economic status.

A cross sectional comparative study was carried out on 91 specimens of healthy human muscle tissues to compare the effect of different types of preservatives at different time intervals on the quality and quantity of DNA extracted from human muscle tissues. It was conducted in the clinical pathology department, Faculty of medicine-Suez Canal University, Ismailia, Egypt, human muscle tissues were collected from two patients admitted to Suez Canal University Hospital for surgical amputation of a part of a limb.

In the present study, cold storage at -20ºC and three preservatives (NaCl, DMSO, Ethanol 70%) were used to preserve human muscle tissue in two situations: room temperature (15-20ºC) (first experiment) and oven incubated at 37 ºC (second experiment). DNA was extracted from the tissue after preservation periods at days 7, 14 and 28. The 37 ºC temperature was used in this study to simulate the conditions which are expected at mass disaster sites in tropical climates. Given that DNA degrades more quickly at higher temperatures **(Alaeddini et al., 2010)**, this temperature represents the worst case scenario so that if a preservative is successful at yielding DNA at this temperature, it is likely that it will also work at lower temperatures **(Allen-Hall and McNevin, 2012).** Moreover, the present study was conducted on human muscle tissues. While most of the previous studies used non-human tissues **(Amos and Hoelzel, 1991; Seutin et al., 1991; Arctander and Fjelds, 1994; Dawson et al., 1998; Williams, 2007; Gaither et al., 2011; Michaud and Foran, 2011)**, few studies were done on human tissues **(Caputo et al., 2011; Allen-Hall and McNevin, 2012)**.

In the present study, it was found that all preservatives were capable of retaining DNA up to 4 weeks in both room temperature (15 to 20 °C) and 37ºC. At room temperature, DNA concentrations which were extracted from all samples declined over time which was also detected by **Allen-Hall and McNevin 2012** and **Shahzad et al., (2009).** When the experiment was repeated at 37ºC incubation (second experiment), DNA concentrations, which were extracted from samples preserved in Ethanol 70% and NaCl increased overtime, while there was no constant trend of DNA concentrations extracted in cases of DMSO and -20ºC preservation. The increase of extracted DNA concentrations over time at 37 ºC might be due to more lysis of muscle tissue with time as heat help in processing and grinding of tissue so more DNA can be extracted **(Allen-Hall, 2011)**. Dry table salt (NaCl) as well as heat cause rapid tissue dehydration leading to rapid tissue

preservation **(Caputo et al., 2011; Allen-Hall, 2011)**. Over time both intracellular and extracellular fluids are decreased due to high temperature and NaCl which makes DNA become more concentrated leading to overestimation of DNA. This explanation was adopted by **Allen-Hall, (2011)** and could also explain the increase in DNA concentrations in the present study. **Allen-Hall and McNevin, (2012)** compared the following tissues preservatives: NaCl, DMSO, EtOH, EtOH–EDTA, DNA-Gen and DNAgard on human muscle tissue and concluded that all of them were suitable for storage of tissue at 35ºC for 4 to 28 days. They also found that samples preserved in NaCl showed increased DNA yield over time. The oven dried samples produced high DNA yields. The mean DNA increased from day7 to 14 and decreased to day 28. In DMSO Sample, the DNA quantity decreased from day 4 to day7, but it increased from day 7 to 14. Then it decreased again from day 14 to day 28 **(Allen-Hall and McNevin, 2012).** The results of the present study are in agreement with the results of Allen-Hall and McNevin, as DMSO samples did not show apparent trend in both studies, while NaCl showed increased concentrations in both studies. **Allen-Hall and McNevin, (2012)** also detected that ethanol 70% preserved samples yielded more DNA than baseline concentrations, which was observed also in the first experiment in the present study and DNA concentration increased over time in ethanol 70% preserved samples which was observed in the 2nd experiment in the present study. This can also be explained by the fact that ethanol evaporates easily and removes water from the sample, which in turn denatures proteins and enzymes **(Seutin et al., 1991; Flournoy et al., 1996)** which also might overestimate the concentration of extracted DNA in addition to the previously explained effect of heat in the 2nd experiment.

NaCl causes a change of ionic concentration within the tissue and desiccation leading to preservation **(Mukaida et al., 2000; Michaud and Foran, 2011)**. A study for long-term room temperature preservation of corpse soft tissue (muscle) was performed by **Caputo et al., (2011)** using solid sodium chloride (salt) at room temperature as one of the methods of preservation. They found that the DNA quantities were similar up to one year and NaCl preservation enabled to obtain high quality genetic profiles. Their results agree with the present study concerning NaCl effectiveness in preserving human muscle tissue in room temperature. However higher temperature (37 ºC) could be responsible for DNA degradation in the second experiment. **Nagy, (2010)** does not recommend NaCl to be used for fresh tissue preservation and consider silica beads to give better results.

In the present study all methods of preservations in the first experiment (-20ºC as well as preservatives at room temperature) retained DNA with no degradation up to the 4 weeks. In the second experiment (-20ºC as well as preservatives at 37ºC), incomplete degradation was found in DNA which was extracted from the samples preserved in NaCl and complete degradation of DNA extracted from the samples preserved in Ethanol 70% at day 28. Otherwise no degradation was found in samples preserved in

DMSO & -20ºC. This degradation might be related to high temperature as DNA degrades more rapidly at higher temperatures **(Alaeddini et al., 2010).** These results indicate that DMSO & -20ºC are the best methods that protect DNA from degradation up to 4 weeks.

Allen-Hall and McNevin, (2012) in their study found that DMSO and Ethanol (70%) showed no degradation while NaCl produced degradation of some samples. The present study agreed that DMSO is the best in higher temperatures as it showed no signs of degradation. Ethanol preserved samples showed full degradation in the present study, which was not found in **Allen-Hall and McNevin, (2012)** study and that might be due to the higher temperature we used (37ºC oven drying) which degrades DNA rapidly. Different extraction methods, different methods of sample collection and DNA profiling were not performed in the present study. It is worth mentioning that extraction methods which were used, could greatly affect the success rate of the used preservation method **(Straube and Juen, 2013).**

Ethanol penetrates cellular membranes rapidly and inactivates nucleases **(King and Porter, 2004).** In a study conducted by **Gaither et al., (2011)** to compare between (ethanol) EtOH 95% and salt-saturated DMSO (SSD) as preservatives of coral specimens, EtOH-preserved specimens yielded more DNA; however, SSD produced higher molecular weight DNA and some EtOH-preserved specimens showed degradation **(Gaither et al., 2011).** This agrees with the results of the present study that EtOH 70% yielded more DNA than DMSO and that DMSO is better than ethanol regarding the quality of extracted DNA at 37ºC, but at room temperature neither of them showed degradation. However, in the present study the duration of preservation was shorter in the present study (28 days vs. 28 months). The results for ethanol are varied and the factors that determine the effectiveness of ethanol as a preservative are concentration, the type of tissue and the amount of time required for storage (**Allen-Hall, 2011)**. **Michaud and Foran, (2011)** detected that alcohols at room temperature were good for short-term storage of tissues, which is suitable for most forensic case scenarios and agree to the results of the present study, as no degradation was found in samples stored in 70% ethanol for up to one month at room temperature. Degradation was observed when 70 % (v/v) ethanol was used to store avian tissue **(Seutin, 1991)** but not when used to stored molluscan tissue **(Dawson et al., 1998).**

Refrigeration was considered less effective than DMSO solution by **Michaud and Foran, (2011)** which was also detected in the present study; as refrigeration yielded less DNA than baseline, DMSO and ethanol 70%. Samples preserved in -20ºC retained DNA with no degradation. **Michaud and Foran, (2011)** used decomposing pig tissues which might have affected their results as DNA degradation have already started before preservation of samples, while fresh human muscle tissue was used in the present study. In the present study freezing did not give the best results which may be due to thawing and refreezing of samples preserved in -20ºC. Freezing of tissue might induce ice crystals which destroy the integrity of cell membrane leading to release of endogenous enzymes

which destroys DNA after thawing process **(Mazur, 1970; Carpi et al., 2011).** Moreover equipment used for freezing can't be controlled all over the day accurately; power supply might be cut suddenly which proves that freezing is not always the best applicable method for tissue preservation.

The DMSO preservative is an aqueous solution that removes water from tissues. It enhances the absorption of other solutes into the cell; it can be considered an enhancing vehicle for other preservatives **(Kilpatrick, 2002).** SSD has several practical advantages over EtOH in being non-flammable and relatively nontoxic and is therefore much easier to transport and store **(Gaither et al., 2011). Kilpatrick (2002)** found that the DMSO preservative (20 %, pH 7.5) produced high molecular weight DNA and successful PCR products from mouse livers after two years storage at room temperature. **Seutin et al. (1991**) stored avian tissue (heart, muscle, liver and brain) in DMSO preservative (20%, pH 8.0) and produced results that were comparable to freezing after storage at room temperature for up to 24 weeks.

LIMITATIONS OF THE STUDY

For economic and technical reasons, PCR and Genetic profiling were not performed in this study, so this is a preliminary study which gives an overview on the used preservatives and their ability to preserve DNA for short durations.

CONCLUSION & RECOMMENDATION

According to the present study, it concluded that DMSO is a successful method of preservation of DNA in human muscle tissue up to 4 weeks in both room temperature and 37 ºC. While NaCl and Ethanol 70% are successful methods of preservation of DNA in muscle tissue up to 2 weeks only in 37 ºC, and up to 4 weeks in room temperature (15-20ºC).

We recommend that this work be repeated with larger sample size and on different human tissues. Larger sample size is also recommended>

ETHICAL APPROVAL

This study was approved by the Research Ethics Committee Faculty of Medicine, Suez Canal University

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الملخص العربى

تـأثيـر مـواد حـافظة مختـلفة لأنـسـجة العضـلات البـشـرية علـى كـفـاءة وكـميـة الحـامـض النـووي: نـظـرة طـبية شرعية

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***قسم الطب الشرعى والسموم الاكلينكية، ** قسم الباثولوجيا الاكلينكية**

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يعد الاستعراف شئ أساسى فى حالة الكوارث. ويتم هذا الاستعراف بطرق عديده ولكن أهم هذه الطرق هى فحص الحامض النووى لذلك لابد من الحفاظ على هذا الحامض النووى من التكسير لكى تتم عملية الاستعراف بنجاح. وتهدف هذه الدراسة الى الحصول على طريقة فعالة لحفظ كمية وكفاءة الحامض النووى بالانسجة البشرية حتى تستخدم فى الاستعراف فى حالات الكوارث وهذا الهدف يتحقق من خلال عقد مقارنة بين تاثير مواد حفظ مختلفة (داي ميثيل سلفوكسيد ، ايثانول 70%، كلوريد الصوديوم و درجة حرارة – 20 ºم) على كمية وكفاءة الحامض النووى المستخلص من أنسجة عضلات بشرية في دراسة تجريبية . وقد تم أخذ عينات العضلات من مرضى محجوزين فى مستشفى جامعة قناة السويس لاجراء عملية بتر لاحدى أطرافهم ، من الجزء الصحى من الطرف المبتور بعد أخذ موافقتهم وموافقة معتمده من لجنة أخلاقيات البحث العلمى بكلية الطب جامعة قناة السويس. وقد تم حفظ عينات من العضلات البشرية في مواد الحفظ السابق ذكرها لمدة 4 أسابيع في درجة حرارة الغرفة (15-20 ºم) وقد تم استخراج الحامض النووي من العينات المحفوظة بعد 7، 14، 28 يوم. كما تم تكرار التجربة مع حفظ العينات في درجة حرارة 37ºم .وقد أوضحت الدراسة أن جميع وسائل الحفظ المستخدمة استطاعت الحفاظ على الحامض النووي طوال مدة الدراسة.وقد كانت اعلى تركيزات للحامض النووى المستخلص في جميع مراحل الدراسة كانت العينات المحفوظه فى ايثانول 70% في كل من درجة حرارة الغرفة ودرجة 37 ºم ، يليها داي ميثيل سلفوكسيد في حالة الحفظ في درجة حرارة الغرفة و كلوريد الصوديوم في حالة الحفظ في درجة 37ºم . جميع وسائل الحفظ المستخدمة قد حافظت على الحامض النووي بدون تكسير في التجربة الأولى (الحفظ في درجة حرارة الغرفة) بينما أظهر الحامض النووي المستخرج من العينات المحفوظة في كلوريد الصوديوم وايثانول 70% والمحفوظة في درجة حرارة 37 ºم تكسيرا ملحوظا في الحامض النووي المستخلص في اليوم الثامن والعشرين. لذلك استنتجت الدراسة أن داي ميثيل سلفوكسيد هى وسيلة ناجحه لحفظ الحامض النووى بأنسجة العضلات البشرية تصل لأربعة أسابيع، بينما ايثانول 70%و كلوريد الصوديوم هى وسائل ناجحه لحفظ الحامض النووى بأنسجة العضلات البشرية حتى أسبوعين فقط فى درجة حرارة 37 ºم وأربعة أسابيع فى درجة حرارة الغرفة (15-20ºم).