POSTMORTEM DETECTION OF LIVER CHIMERISM AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ADULT MALE MICE

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ABSTRACT

Introduction: Postmortem personal identification is based on the extraction of deoxyribonucleic acid (DNA) from human remains or different human materials. There are some reports proving that various tissues may differ genetically and do not always have the DNA profile of the person from whom they originate due to mutation or chimerism. **Aim of the work:** The work aimed at the postmortem detection of the possible change in DNA fingerprint after allogeneic hematopoietic stem cell transplantation in liver tissue samples. **Materials and Methods:** One hundred twenty eight adult male mice weighing 25g. DNA fingerprint first was done from blood samples. After 3 months from interference, postmortem DNA fingerprinting was done again from liver tissue. **Results:** Fluorescent stem cells were detected postmortem in liver tissue of 30 mice by percentage of 93.75%**.** The change in DNA profile was detected postmortem in 20 mice in liver tissue samples by a percentage of 62.5%. **Conclusion:** DNA chimerism, after allogeneic hematopoietic stem cell transplantation, can be detected postmortem in the recipient's liver. **Recommendations:** It's recommended to study the postmortem detection of DNA chimerism in organs other than the liver to compare the percentage of chimerism detection.

Key words: Postmortem; Liver; Chimerism; Allogeneic Hematopoietic Stem Cell Transplantation.

1. INTRODUCTION

Personal identification for patients who have undergone allogeneic hematopoietic stem cell transplantation (HSCT) is considered a major challenge **(Santurtún et al., 2017).** Medicolegal identification of an unknown person consists of the comparing individualizing features from antemortem information with postmortem data from the unidentified corpse **(Angelis et al., 2016).** This is not only important for the criminal and civil legal proceedings, but also, for the ethical consequences and the human right of being buried with his/her own name and mourned by relatives after death **(Cattaneo et al., 2010)**. Based on the hypothesis that all human body cells have a consistent DNA profile, different types of DNA-containing tissue and fluids can be used to find a match **(Jacewicz et al., 2013).**

Allogeneic hematopoietic stem cells transplantation (allo-HSCT) has been widely used to treat patients with several illnesses, but the biological materials of those recipients may cause problems in personal identification due to what is called "chimerism" **(Li et al., 2014).** Chimerism is defined in transplantation medicine as the coexistence ofcells of, both, donor and recipient origin **(Ten Hove et al., 2007).** In previous studies, blood, hair samples and buccal swabs are studied as sources of DNA profiling after hematopoietic stem cell transplantation **(Hong et al., 2007; Zhou et al., 2011; Li et al., 2014; Chaudhary et al., 2015; Santurtún et al., 2017)** The partial chimerism is detectable predominantly in the

hematopoietic system developed by transfusion or transplantation of allogenic hematopoietic stem cells **(Milde et al., 1999).**

This experimental work aims at studying the possibility of postmortem detection in DNA chimerism in liver tissue, after allogeneic hematopoietic stem cell transplantation.

2. MATERIALS AND METHODS

2.1. Sampling and experimental design

This study was done after the acceptance of Institution Review Board (IRB) of faculty of Medicine, Zagazig University.

One hundred twenty eight adult male mice (32 mice in each group) weighing 25g obtained from the animal house of faculty of Medicine, Zagazig University were included in the study.

The experiment was performed in accordance with the Guide for the care and use of laboratory animals.

Group I (Control group): It was divided into three subgroups: **Group Ia,** it was the negative control subgroup in which the mice didn't take any drugs. **Group Ib,** mice receive 2 intraperitoneal (i.p.) injections of Busulfan (35mg/kg) 24 hours apart, then Cyclophosphamide (100mg/kg) intraperitoneal injection 24 hours after last Busulfan dose **(Nevozhay & Opolski, 2006) Group Ic,** It was the control group injected with hematopoietic stem cell without immunosuppression.

Group II (Hematopoietic stem cell transplanted group):

Hematopoietic stem cell transplantation was done after immunosuppression. Mice recieved 2 i.p. injections of Busulfan (35mg/kg) 24 hours apart before stem cell injection by 4 days, then cyclophosphamide (100mg/kg) i.p. injection 24 hour after last Busulfan dose **(Nevozhay & Opolski, 2006).** Hematopoietic stem cell transplantation then done.

In all groups, DNA profile was done from blood before hematopoietic stem cell transplantation from all mice. Three months after interference, all mice will have DNA profile again done from postmortem liver tissue.

Blood samples: 0.5 ml blood was collected from orbital sinus of mice in the 1.5 ml micro centrifuge tubes **(Parasuraman et al., 2010).** Postmortem tissue samples were taken by a surgical incision from the liver **(Picazo MG & García-Olmo, 2015).**

2.2. Hematopoietic stem cell transplantation

Ten ml blood was isolated from donor mice by using ficoll gradient precipitation method.

Hematopoietic stem cells were separated using separating column to isolate Cd34 cell (HSC) from MSC the cultured in complete culture medium. After trypsinization, staining of cells was done by PKH26 red fluorescent cell linker. Then the cells were injected IV in the tail vein of the mice.

2.3. Fluorescent microscopic examination

Fixed liver tissue was sectioned into very thin (5 micrometers) sections using a microtome; the sections were examined under fluorescent microscope.

2.4. Deoxyribonucleic acid profiling:

Deoxyribonucleic acid extraction was done according to the manufacturer's instructions in **G-spin™ total DNA Purification Kit *(Fermentas, EU, 2014).***

Reaction mixtures were subjected to the cycling program in a DNA heated lid thermal cycler. The PCR conditions were 95˚C for 3 min, 95˚C for 2 min, 30 cycles of (94˚C for 30

s, 50-52˚C for 30 s and 72˚C for 1 min) a final step of 72˚C for 5 min.

Polymerase chain reaction (PCR) products were separated on 3% agarose gels. The gel was stained with ethidium bromide and photographed under UV trans-illumination.

Table (1): Primers used in the multiplex reverse transcriptase polymerase chain reaction.

Gene	Primer sequence (5`→3`)	Size (bp)
18-3	F: TCTTTCTCCTTTTGTGTCATGC R:GTTTCTTGCTAAATAACTAAGCAAGTGAACAGA	281–313
4-2	F: AAGCTTCTCTGGCCATTTGA R: GTTCATAAACTTCAAGCAATGACA	217–248
6-7	F: AGTCCACCCAGTGCATTCTC R: GTTTCTTCATGTGGCTGGTATGCTGTT	333–515
9-2	F: GGATTGCCAAGAATTTGAGG R: GTTTCTTTCCTGAGTTGTGGACAGGGTTA	318–360
15-3	F: TCTGGGCGTGTCTGTCATAA R: GTTTCTTTTCTCAGGGAGGAGTGTGCT	157–222
6-4	F: TTTGCAACAGCTCAGTTTCC R: GTTTCTTAATCGCTGGCAGATCTTAGG	276–311
12-1	F: CAAAATTGTCATTGAACACATGTAA R:GTTTCTTTCAATGGTCAAGAAATACTGAAGTACAA	222–259
5-5	F: CGTTTTACCTGGCTGACACA R: GTTTCTTGGTTTAAAACTCAATACCAAACAA	258–298
X1	F: GGATGGATGGATGGATGAAA R:GTTTCTTAAGGTATATATCAAGATGGCATTATCA	357–442

3. RESULTS

3.1. Fluorescent microscopic examination

Stem cells weren't detected in postmortem liver tissue of any mice of group I in all subgroups of group I: group Ia, group Ib and group Ic → percentage 0% but they were detected postmortem in liver tissue of 30 mice of group II by a percentage of 93.75% **(Figure 1 and Table 2)**.

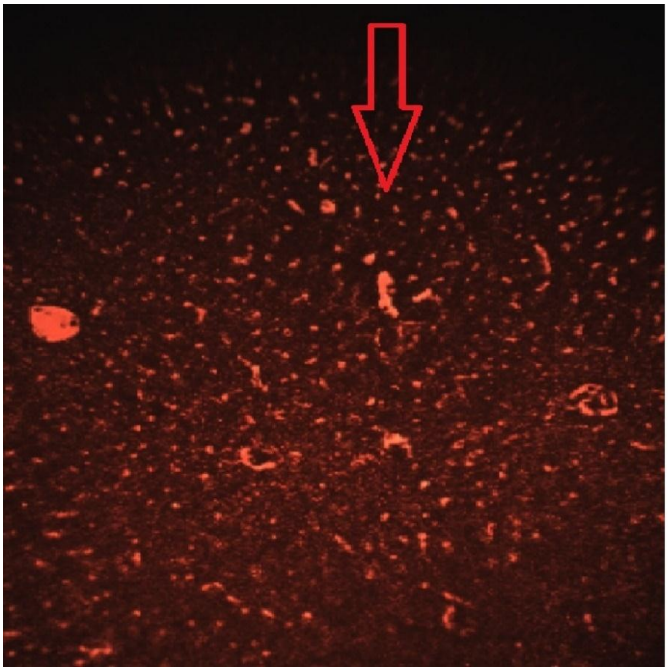

Figure (1): Fluorescent stem cells in postmortem liver tissue (arrow).

Table (2): Comparison of the percentage of fluorescent stem cells presence in postmortem liver tissue in adult mice, n=32.

Parameter	Group I (Control groups)	Group II	X^2
Fluorescent stem cells percentage	0%	93.8%	117.55 ***

P < 0.001 ***

3.2. Deoxyribonucleic acid profiling

There is no detected change in DNA profile in blood samples, hair, buccal swabs and postmortem liver tissue in any mice in group I: Ia, Ib and Ic → percentage 0%**.** The change in DNA profile was detected in 20 mice postmortem in liver tissue samples by a percentage of 62.5% **(Figures 2, 3 and Table 3).**

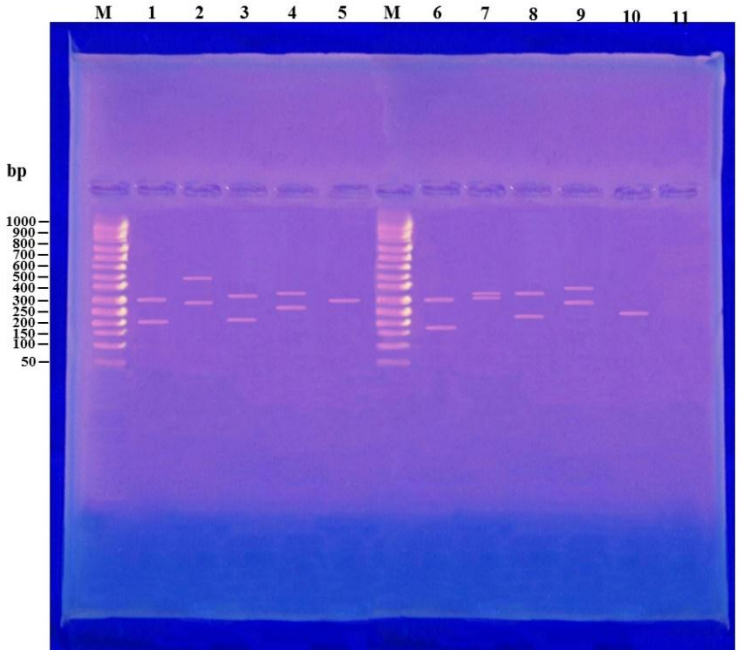

Figure (2): Change in deoxyribonucleic acid profile in postmortem liver tissue samples after hematopoietic stem cell transplantation.

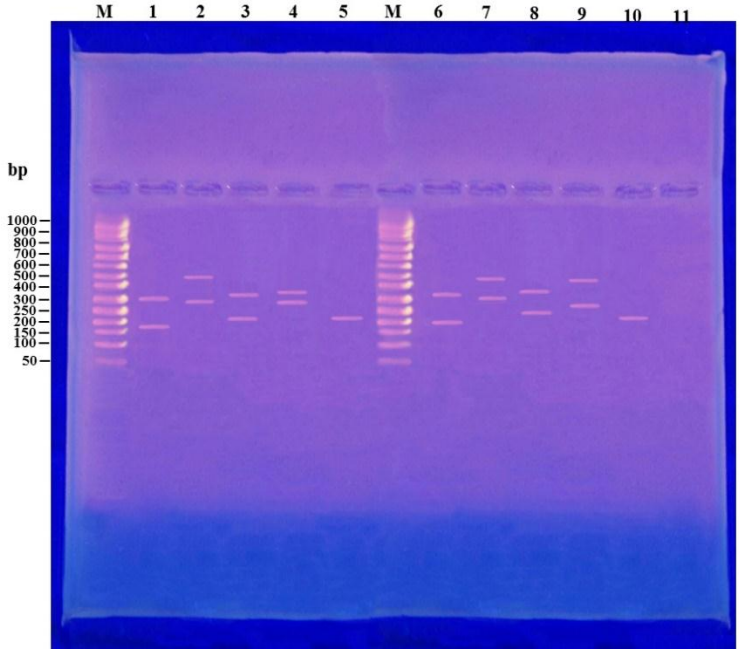

Figure (3): Change in deoxyribonucleic acid profile in postmortem liver tissue after hematopoietic stem cell transplantation.

Table (3): Comparison of the percentage of DNA profile change in postmortem liver tissue samples in adult mice, n=32:

Parameter	Group I (Control groups)	Group II	X^2
Postmortem liver tissue	0%	62.5%	71.111 ***

P < 0.001 ***

4. DISCUSSION

The assumption that all the cells in the human body have the same DNA profile which constitutes the basis for comparative analysis in forensic genetics was discovered to be wrong when tissues of recipients after allogeneic stem cell transplantation are investigated **(Dauber et al., 2004; Andréasson et al., 2006).**

This work aimed at the postmortem detection of the possible change in DNA fingerprint after allogeneic HSCT in liver tissue samples. Many studies not only confirmed the mobilization and presence of hematopoietic cells in liver tissue after HSCT, but also proved their differentiation into hepatocytes **(Jang et al., 2004; Zhan et al., 2006). Abkowitz (2002)** revealed donor-derived cells in almost all tissues of recipients after allo-HSCT. They found that human hematopoietic stem cells could become skin, gut, or liver cells**. Körbling et al. (2002)** examined biopsy specimens from the liver, gastrointestinal tract, and skin were obtained from 12 patients who had undergone transplantation of hematopoietic stem cells and proved showed evidence of complete hematopoietic donor chimerism in biopsy specimens**. Santurtún et al. (2017)** concluded in their study that whatever the cellular source of HSCT donor's DNA might be, mixed chimerism with both donor's and host's DNA profiles can be detected, and this must be taken into account when performing DNA profiling of cases potentially involving persons who **had undergone HSCT.**

Mosaad (2014) stated that hematopoietic stem cells are determined travelers. Migration of hematopoietic stem cells through the blood, across the endothelial vasculature to different organs and to bone marrow is termed homing. Donor cells not only can reach liver after hematopoietic stem cell transplantation but also they can transform to hepatocytes **(Moon et al., 2009).**

5. CONCLUSION

Hematopoietic stem cells can be detected postmortem by a percentage of 93.75% in liver tissue after hematopoietic stem cell transplantation. The change in DNA profile postmortem in liver tissue samples by a percentage of 62.5%.

6. RECOMMENDATIONS

As hematopoietic stem cells can be detected in liver tissue after hematopoietic stem cell transplantation, other types of samples have to be studied for this purpose.

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الكشف بعد الوفاة عن خيمرية الحمض النووى في خلايا الكبد بعد زرع الخلايا الجذعية المكونة للدم الخيفى في ذكور الفئران البالغة

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ان الاستعراف الشخصي بعد الوفاة يعتمد علي استخراج الحمض النووي من البقايا البشرية أو المواد البشرية المختلفة. وقد أثبتت بعض الدراسات أنه من الممكن أن يوجد اختلاف في تحليل البصمة الوراثية بين أنسجة الجسم المختلفة لنفس الشخص نتيجة الطفرة الوراثية أو خيميرية الحمض النووي.

هدف الدراسة: كان هدف هذا البحث دراسة احتمال تغيير البصمة الوراثية في عينات الكبد بعد الوفاة بعد زرع الخلايا الجذعية المشكلة للدم الخيفي في ذكور الفئران البالغة.

الطرق والأدوات: تم اجراء البحث على 128 من ذكور الفئران البالغة التي يزن كل منها 25 جرام في قسمى الطب الشرعي و السموم الإكلينيكية و الكيمياء الحيوية (معمل زراعة الخلايا الجذعية)- كلية الطب– جامعة الزقازيق. تم عمل تحليل البصمة الوراثية من الدم لكل الفئران قبل زراعة الخلايا الجذعية المشكلة للدم الخيفي وتم اعادته بعد 3 شهور بعد زراعة الخلايا الجذعية من الكبد لمعرفة مدي التغيير في تحليل البصمة الوراثية.

وقد أظهرت نتائج البحث وجود الخلايا الجذعية المشكلة للدم في عينات الكبد بعد الوفاة في 30 فأربنسبة 93.75% وذلك يؤكد أنها خلايا متحركة تنتقل إلي الأحشاء بعد زراعتها في الدم، كما أظهرت حدوث تغيير في فحص البصمة الوراثية بعد زراعة الخلايا الجذعية المشكلة للدم في عينات الكبد بعد الوفاة في 20 فأر بنسبة 62.5%.

الاستنتاج: أن خيمرية الحمض النووي وتغيير البصمة الوراثية تحدث بعد زراعة الخلايا الجذعية المشكلة للدم الخيفي في خلايا الكبد الخاصة بالمريض.

التوصيات: يمكن استخدام عينات من الكبد بعد الوفاة كمصدر لعمل البصمة الوراثية في المرضي الذين يقومون بزراعة الخلايا الجذعية المشكلة للدم الخيفي مع ضرورة البحث عن أنواع عينات بيولوجية جديدة لعمل تحليل البصمة الوراثية