Successful Use of STR Analyses Using Iraqi Match Software for Identification of Unknown Completely Burned Bodies

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ABSTRACT

Background: New simple match software program had been used to solve one of the most important gradients cases that facing medical and biological staff in DNA Typing Department of Medical-Legal Directorate (MLD) in Iraq due to explosions which lead to the inability of identification of disfigured badly burned human cadavers.

Objective: To focus the light on the importance of DNA Typing in human identification in explosion incidents happened in Iraq and the importance of using simple software program created in MLD /Baghdad to ease and shortening the time of DNA result interpretation stage of analysis.

Methods: Fifteen burned cadavers were the outcome of an explosion happened in Baghdad city in 24/11/2008 in a mini bus related to Iraqi Trade Ministry. All were referred to MLD, 2 of them known to be males and 13 females house officers, 7 of those burned bodies were completely disfigured and hardly to be identified by their families. Morphological, personal belongings examination and DNA finger printing were used to identify severely bad burned cadavers.

IT Unit had been asked to create software program to ease the comparison of DNA profiles between missed victims and their alleged families in such case. Then genetic profiles had been loaded to the newly created match software program called Missing DNA Samples Comparison Program.

Results: We could identify all the unrecognizable victims in the Trade Ministry minibus explosion in a very short time with the aid of software program which is simple, easy, fast, read in Arabic language using infinite numbers of genetic profile for effective match between the victims and families' profiles. All victims had been satisfactory given to their families.

Conclusion: Despite of the shock, sadness, exhaustion and pain of victim's families, they were highly cooperative and consistence to know the exact cadaver that belonged to them by DNA fingerprinting technique in order to be buried in respectful manner with comfort feeling. In each heavy explosion, it should be mandatory in each forensic casework to withdraw blood reference sample from all victims' families beside representative sample of bodies to avoid body exchange by ignorant family human mistake. New Missing DNA Samples Comparison Program created in MLD makes matching of DNA profiles much easier and shorter time than manual method.

Keywords: DNA fingerprinting, Explosion, Human identification.

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Explosions in Iraq was so frequently occurring after USA invasion at 2003 due to violence and deterioration in security situation which lasted many years after, associated with many tragedies. Most of such series of explosion were happened in Baghdad resulted in many severely burned disfigured dead bodies or parts of human remains.

Explosion could be small, harms few numbers of people or massive, affecting buildings and thus large numbers of humans might be injured. It might be happened by containers at roadsides or in vehicles or bomb suicide and may occur by mortars.

However, in historical cases, missing persons', mass disasters and disfigured cadavers, identification is usually based on morphological, anthropological and

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archeological examination of cadavers or human remains in addition to the use of forensic odontology and personal belongings findings[1].

DNA analysis has become an important tool in forensic and anthropological casework because of its accuracy, highly discriminating and unique to each individual and the ability of matching of DNA profile of the skeletal remains with the DNA profile of their family members[1,2,3].

Analysis of nuclear DNA –STR testing has been proven to be valuable for identifications in mass fatality incidents and in cases of high degradation of human remains such as plane crashes, terrorist attacks, natural disasters, armed conflict, or any other case where traditional methods of identification are insufficient[4].

Following such events or incidents, it is desirable to group together body parts from individuals when fragmentation has occurred ultimately to identify the deceased. If the time between death and recovery of the body is short then muscle tissues provide a rich source of DNA[2,5], while in some cases when human remains or badly burned cadavers are found, bone and teeth are excellent sources of DNA for human identity testing through uses of STRs or mitochondrial DNA (mt DNA)[3].

Therefore, based on what have been mentioned above, in any explosion happened, after morphological and personal belongings examination of dead by forensic pathologist and cooperation of relative families, identification of their beloved victims were tried always. The biological staff in morgue of MLD are keen to collect the suitable representative sample from the bodies and human remains whether tissue or bones to be in good condition and enough amount for DNA Typing. Collection of blood samples from families are mandatory after each explosion.

Relatively specialized techniques are required when DNA in the cellular material are degraded, particularly for the extraction of DNA from bone, when the bones have been exposed to adverse environmental conditions or severe environmental insult such as high temperatures[3,6,7].

In MLD, variable documented confidential methods for DNA extraction are used according to the type, amount and status of the referred sample. Again variable DNA – Typing kits and though PCR programs are used like Identifiler, Minifiler and Powerplex STR multiplex.

In any crime or accident, after completion of DNA Profiles of representative and reference samples, comparison between both is usually done manually in criminology lab of DNA Typing Department. This method of comparison is so easy when limited number of DNA profiles are included in the forensic casework, whereas it becomes difficult if there are large number of victims' DNA Profiles and their families like in explosion incidents.

In such concern, it was well known that the wide spread of computers at home and in the workplace has increased dramatically since personal computers became available in the mid-1980s associated later with some interesting parallels between computer technology revolution done by Microsoft Corporation, and the timing for forensic DNA typing field advancements. Furthermore, for high numbers of samples or data, data assessment and interpretation of STRs may require more than 50% of resources to deliver final results for all samples. DNA databases can also play an important role in helping to identify missing individuals and aiding mass disaster reconstruction following incidents resembling explosion like a plane crash or terrorist activity. In these cases, the software systematically compared each available STR genotype with every other genotype. It is preferable to use parentage analysis of the genotype derived from the personal effect with the genotypes derived from family relatives.[8,9]. In foreign countries, there were many software created, some are lab-developed solutions, such as Excel

spread sheets and some are effectively cost to pay.

DNA View®, Gene marker® HID software, Mass Disaster Kinship Analysis Program (MDKAP), M-FYS is for Mass Fatality Identification System. GPC (Genetic Profile Comparisons) module integratedin SQL-LIMS system to perform "victims against victims" and "victims against references" and a lab-developed software tool (PATPCR-v 2.52) are examples for worldwide and national software programs that ease the result interpretation of DNA Profiles by pair-wise comparisons which require bioinformatics capability to search all-against-all large STR database of DNA profiles beside forensic mathematical expert support[10].

Accordingly, after war in 2003 and later when DNA Typing Department was established practically in MLD /Baghdad at the end of 2007. It was difficult to communicate with experienced personnel abroad because of unstable security situation and to pay for the above mentioned software programs. Therefore, IT Unit in MLD had been asked to create and develop a simple software program that enable the staff in DNA Typing Department to compare the DNA profiles resulted from representative samples of victims due to explosion with that of DNA profiles resulted from reference samples of the alleged families and relatives. Fortunately, IT Unit was successful in creating such program which had been examined and used for the first time in Iraqi Trade Ministry minibus explosion described here in this study.

Methods

Incident history: Full information about the incident revealed that in 24 of November 2008, a mini bus was on its way morning to Ministry of Trade carrying 15 persons, 14 of them were house officers of the ministry and the last one was the driver, suddenly explosion was happened by adhesive packaging to the minibus. However, the bus was destroyed and completely burned so as the people inside, all were referred to the MLD / Baghdad in the same day. Their families were rushed to the morgue. There were 13 missed females and two missed males including the driver.

Fifteen families were allowed to see and check morphologically the victims and the personal belongings for purpose of identification in order to bury them according to the traditional, respectable funeral Iraqi custom.

Morphological or external examination had been done in the morgue depends on facial appearance, height, weight, sex and victims' clothes and personal belongings, all these points helped the forensic pathologist and the families to identify 8 of the cadavers, one of those cadavers was identified as a male driver and 7 of them were identified as female house officers. for example: one of the female was recognized and identified because of her extreme obesity while another female was recognized and identified by her necklace and jewels.

All were considered to be known and had been given to their families directly with death certificates.

The rest of bodies were seven cadavers out of 15. They were severely burned and completely disfigured, thus their recognition or identification by morphological examination was so difficult and impossible, and they were considered to be Unknown, (Table 1).

Table 1: The outcome of Known and Unknown Iraqi cadavers after morphological examination from Trade Ministry minibus explosion in Baghdad / 2008.

Type of body	Number
Known bodies/ morphologically	8
Unknown bodies/ morphologically	7
Total number of bodies	15

At this time the DNA finger printing was the only best technique should be used to identify the seven (7) left badly burned cadavers.

DNA Typing Technique: Victim's names and their families (fathers, mothers, sons and daughters) were listed in an official order of police office with the request of identification, this order with the referred families were received by DNA department in MLD/Baghdad. Six of the families sought for female gender victims and one family sought for male gender victim. The following steps were followed:

-labeling: Each obtained sample from each cadaver had the same number and symbol of the cadaver itself. i.e. (code number). FTA cards were labeled and named by the relatives.

-Sample collection from bodies: One bone and six tissue samples were collected. These samples were clean, not burned, not contaminated and representative, with enough amount as a source of DNA from most of the cadavers. Bone sample was obligatory taken from completely burned body (when soft tissue sample was un available).

-FTA card for families: A reference samples of blood were withdrawn from the relatives of the victims and blotted on FTA cards i.e. father, mother, son or daughter.

One of the female victims was unmarried and her parents were died (orphan), therefore no reference sample could be obtained from her family, so that we asked her relative to bring some of victim's belongings like her hair comb or tooth brush to be used as a reference sample. Fortunately, 4 hair follicles could be obtained from her comb.

-DNA Profile: Collection of samples were done by the staff members of the Samples Collection Unit of Missing Department. Both representative and reference samples were sent to the DNA typing department.

DNA was extracted from tissue, bones and hair follicles by phenol-chloroform method while DNA from bloodstain on FTA cards was extracted by chelex method. The amount of isolated human DNA was quantified using the ABI Quantifiler™ Human DNA Quantification Kit, (Applied Biosystems), ABI 7900 real time detection instrument (Applied Biosystems), and then suitable dilutions had been done to get appropriate concentration for PCR technique.

Simultaneous amplification of 16 STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818, FGA AND AMELLOGENIE) were done by using the AmpFlSTRIdentifiler™ PCR amplification kit according to the manual recommendation on 9700 PCR instrument (Applied Biosystem) including the positive and negative control. Capillary electrophoreses was used for the detection of the amplified product and the run was conducted with ABI prism 3130xl Genetic analyzer (16 capillaries array system-Applied Biosystem) with the allelic ladder, positive control and negative control. The raw data were collected by Data collection software and then analyzed by Gene mapper software. All DNA Profiles were female in.

-Matching Software issue (Missing DNA Samples Comparison Program):

Matching between the DNA profiles had done by newly developed software created by staff member of IT Unit. The program is in Arabic language[(1)], (Figure 1), it contains 3 phases, the first phase is to load the code numbers of the victims with their DNA profile, (Figure 2).

Second phase is to load the name of reference samples with their DNA profiles, (Figure 3).

Then in the third phase, the software matched each DNA profile of the victims with all DNA profiles of the reference samples and then the result was represented as the percentage of compatibility which was limited to (Zero, 50 and 100) only.

For example, 5 of the victims' DNA profile were compatible 50% with their corresponding 5 of reference samples and one female bone DNA profile was compatible 100% with her hair reference sample, (Figure 4).

Figure 1: Software Program for comparison between DNA Profiles of representative and Reference samples in Missing cases i.e. Results Interpretation

Figure 2: Loading DNA profiles of representative samples.

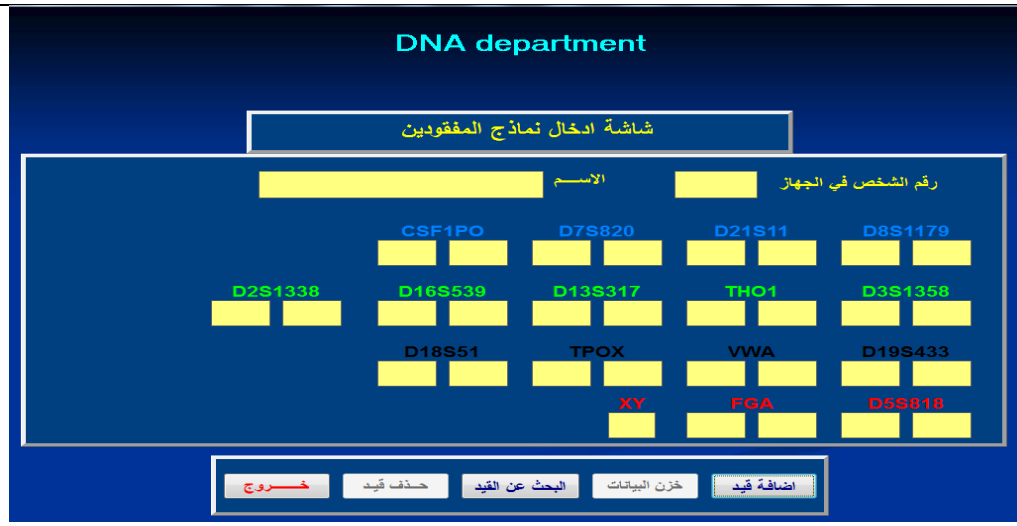

Figure 3: Loading DNA profiles of references samples.

Figure 4: Result interpretation, percentage of compatibility between representative and references samples.

Results

Now 6 cadavers out of 7 were identified and become Known whereas 1 DNA profile didn't match any of reference samples, this female cadaver remained Un Known. In another hand one family remained asking about the fate of its beloved missing who was male in gender.

Therefore, this result proposed that switching of cadavers had been occurred between 2 families. So, we thought that an exchange was happened by mistake between the male cadaver instead of one of female cadavers during preliminary morphological examination in the morgue, this mistake was proven to be truly happened at that time.

Discussion

From above mentioned results, 6 female bodies out of 7 were identified by genetic profiles and return back to their families with death certificates.

But in contradiction to the claiming of families, the outcome revealed that all seven Un Known cadavers were females in gender, not as expected (6 females and 1 male), therefore one of the female DNA profile did not match any profile of the present families so she was left Un Known.

Thus, to solve this problem, all the eight (8) families who recognized their victims morphologically in the preliminary stage of identification at morgue were recalled again to visit MLD to withdraw blood sample from them for DNA Typing. Three families were responded cooperated, and agreed to provide blood sample. We could easily know the family that take wrongly the male cadaver, because the profile of the left Un Known female matched one of cooperated family perfectly.

Hence, from all the samples (bone or tissues) complete DNA profile were obtained and matched with their relatives' profile (blood samples). The matching software revealed 50% ratio compatibility, in addition all statistical analysis was done manually and all father or mother/son or daughter cases revealed 99.9 PI (paternity index).

The biological staff in DNA Typing Department was so satisfied with their primitive first experience in using 4 hair follicles as a reference sample for a female who was lonely in her life (orphan). The DNA profile that was obtained from the bone sample and was completely matched with the profile from the hair of 100% compatibility ratio in our new created software shown in Figure (1) in Arabic language.

Moreover, we were so satisfied about the use of IT Unit´s software program which can be a nucleus for simple database in missing persons' cases.

The idea behind this software resembles any software created as a valuable tool to manage DNA Data base aiding law enforcement investigations but in simple manner. The technology of forensic DNA databases basically as it is known involves three steps: The collection of known specimens. Analyzing those specimens and loading their DNA profiles in a computer database. Subsequent comparison of unknown profiles obtained from crime scene evidence with the known profiles in the computer database[8].

Although we checked the program before use and although we used the software for the first time in this case, we return back to check each DNA Profile of the missing with the corresponding family manually. It was perfect, simple, accurate, can be trusted, easy, fast, read in Arabic language and may use infinite numbers of genetic profile for effective match between the victims and families' profiles (Father, Mother, son or daughter) or (victim to victim´ s reference sample).

In spite of all these benefits of this new created software there were some draw back in using it in comparison to programs like DNA view, gene marker and emphasis (Note: the last 2 programs were bought and used in mass graves department later after 2013 in MLD /Baghdad). The draw back may be summarized in the following points:

1- It isn't smart enough to use in complicated cases or cases of mass graves or when there are large numbers of victims with no father, no mother or son donors and should the matching done with at least 3 brothers and sisters.

2- It doesn't provide any mathematical calculations like paternity index (PI), sibling proportion and statistical analysis which should be done and depended especially when we deal with population greatly related to each other due to marriage ethics and habits within Iraqi population. In Iraq the marriage between relative is more common.

3- This software cannot draw the family tree which represent the relation between missing persons and family.

4- Inability to load all the data (information and documents) that related to the donors and missing persons like for example in emphasis software.

On the other hand, there were many advantages by using this new created software:

1-Easy for use.

2-Can deal with simple cases i.e. limited numbers of missing persons with the relations between families and their missing persons are not complicated.

3-The most important point is availability because it is homemade software at about near the beginning of DNA Typing Department case works, thus cheap and not expensive like other types of software.

In fact, this case focused on the importance of the DNA finger printing or STR analysis used in MLD/Iraq to solve the major problems of identification following explosions. In addition, DNA Typing Department announced later Dead Investigation Department and Sample Collection Unit of Missing Department to collect biological sample from each family and cadaver in each forensic casework of explosion even if morphologically the corpse has been identified by ignorant family to overcome the problem of body exchange by human error. Furthermore, newly matching software created by IT unit staff in MLD helped us to solve the incident of Iraqi mini bus explosion related to trade ministry, facilitate result interpretation, save the time and efforts. Matching process became easier than the manual one.

Finally, we could identify all the unrecognizable victims in the present study. All victims had been satisfactory given to their families. Such program had been used frequently later in many other explosions happened in Baghdad resolved them quickly and perfectly.

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