Research Journal of Forensic Sciences ISSN 2321–1792
Vol. **12(2),** 1-7, July **(2024)** *Res. J. Forensic Sci.*

The genomic DNA profiling of unidentified dead bodies – A forensic perspective from Himachal Pradesh, India

Naresh Kumar*, Aishwarya Thakur and Hem Raj
DNA Unit, Regional Forensic Science Laboratory, Mandi, Himachal Pradesh, India
nareshkumarbiotech85@gmail.com

Available online at: www.isca.in, www.isca.me
Received 22nd July 2023, revised 14th February 2024, accepted 4th March 2024

Abstract

Deoxyribonucleic acid (DNA) profiling from different samples of unidentified dead bodies is a challenging task in forensic laboratories. These dead bodies are recovered from mass disasters, forest fires, traffic accidents, avalanches, plane crashes, etc. DNA profiling from these dead bodies is essential for law enforcement because concerned relatives of the unknown deceased may later make legal claims. Therefore, it is crucial from a forensic and legal perspective to identify these dead bodies. In this study, the demographic overview and DNA profiling of unidentified dead bodies from Himachal Pradesh, India was assessed for a period of seven years i.e., 2014 to 2020. Different parameters were selected for the demographic study and DNA profiles were generated from different samples viz. blood sample on Flinders Technology Associates (FTA) cards, blood samples on cotton gauze, muscle tissues, hair, teeth, blood samples, skin tissue, bones such as sternum, femur, ribs, jaw, humerus, clavicle, skull, radius, thumb, ulna, tibia, and fingers. It was found among unidentified dead bodies; the most common age groups were 21-40 years (38.2%) with 91.8% male. Most of the unidentified deceased were retrieved in decomposed state (32%). The river was the most common place where dead bodies were retrieved due to drowning (12.8%) and most of them were recovered during the rainy season of Himachal Pradesh i.e., July-September (29.4%). Furthermore, the percentage of DNA profiles from different samples was calculated and it was observed that bones such as thumb, humerus, radius, ulna, clavicle, sternum, ribs, femur, finger and skin tissue, teeth, blood samples on cotton gauzes were suitable exhibits for genotyping from both fresh and putrefied unidentified dead bodies. This study will help medical professionals in collecting and preserving suitable exhibits during autopsies of unidentified dead bodies for complete DNA profiling in forensic science laboratories so that law enforcement agencies can minimize the social and legal burden of the same. Furthermore, a database can be created from the DNA profiles of unidentified dead bodies to determine their identity.

Keywords: Database; DNA profiling; FTA; Unidentified dead bodies.

Introduction

Unidentified dead bodies are those people who have died and whose identity cannot be ascertained by the local public, police and medical experts. Every year, a large number of unidentified dead bodies are found around the world. As per the National Crime Records Bureau (NCRB), New Delhi, a sum of 34592 unidentified dead bodies were found in India during 2015. Indian states like Maharashtra (6185), Tamil Nadu (3739), Karnataka (3533), Uttar Pradesh (3409) reported the highest number of unidentified dead bodies, while Mizoram, Nagaland, Sikkim and union territories viz. Andaman and Nicobar Islands, Dadra and Nagar Haveli, Lakshadweep were the lowest. In Himachal Pradesh, 92 unidentified dead bodies were found in the same year[1]. Unidentified dead bodies are usually recovered from incidents like homicides[2], disasters[3], mass disasters[4,5], trekking[6], wars[7], burning due to traffic accidents[8], forest fires[9], terrorist attacks[10], plane crashes[11], armed conflicts[12] and floods[13], etc. Sometimes these bodies were not discovered by the local people and the police, and therefore they significantly decompose due to burning, scavenging, decomposition, mutilation, etc. over time, making visual identification by clothing and personal belongings impossible[14]. The identification of these bodies would not only assist the family in carrying out the deceased's last rites, but it would also aid in identifying the perpetrators in cases where the mutilation of dead bodies is done on purpose in an effort to erase all traces of identity or make the body easier to dispose of. Human identification from degraded and decomposed samples is challenging for forensic geneticists, forensic pathologists and forensic odontologists[3,5]. The DNA profiling has emerged as gold standard for identifying unidentified dead bodies[15]. DNA profiles can be generated from challenging unidentified cadaver samples using Short Tandem Repeat (STR) technology with statistically significant values and the profiles can be stored in a DNA database. Some countries such as the United States have a "NamUs database" for unidentified remains and missing persons. There is no DNA database in India, however a medical organization viz. All India Institute of Medical Sciences (AIIMS), New Delhi has started keeping DNA records of unidentified dead bodies using the UMID portal[16]. DNA profiles stored in a database of unidentified dead bodies can help establish links between missing persons and the deceased. With the introduction of "The DNA Technology (Use and

Application) Regulation Bill 2019", the DNA database will have a legal basis in India.

Himachal Pradesh is the northernmost state of India in the western Himalayan region. There are twelve districts with a total area of 55,673 sq. km[17]. Being a mountainous state; there are huge differences in climate, weather and geography, which may be one of the reasons for encountering unidentified dead bodies. There is less available literature on demographic data and DNA profiles of unidentified dead bodies in Himachal Pradesh. Keeping this in view, this study was conducted at DNA Division, State Forensic Science Laboratory, Junga, Shimla, Himachal Pradesh to review the demographic data and assess the cause and manner of death of the unidentified dead bodies. DNA profiles were also created from various samples of unidentified dead bodies that could assist medical professionals in collecting and preserving suitable exhibits for DNA profiling.

Material and Methods

Materials: In the current study, 500 samples from unidentified dead bodies were randomly selected and analyzed (Table-1). These samples were received at the DNA Division, State Forensic Science Laboratory, Junga, Shimla between years 2014-20 for DNA profiling through a legal process from various police stations of the state of Himachal Pradesh.

Table-1: DNA profiles in percentage from various samples of unidentified deceased in Himachal Pradesh, India

S. No.	Sample type	Numbers	Percentage of DNA profiles	
			Complete	Partial
1.	Blood sample on FTA card	68	79	21
2.	Blood sample on cotton gauze	16	88	12
3.	Muscle tissue	18	61	39
4.	Hair	05	50	50
5.	Teeth	96	85	15
6.	Blood	63	67	23
7.	Finger	05	80	20
8.	Sternum bone	121	97	3
9.	Clavicle bone	18	100	-
10.	Femur bone	26	92	8
11.	Ribs bone	35	89	11
12.	Jaw bone	01	No profile	
13	Humerus bone	11	100	-
14.	Skull bone	05	No profile	
15.	Radius bone	04	100	-
16.	Thumb bone	01	100	-
17.	Skin tissue	04	100	-
18	Ulna	01	100	-
19.	Tibia	02	50	50
Total		500		

Methods: Genomic DNA Extraction: The genomic DNA from various samples was isolated and purified using three methods: a) DNA purification from FTA cards b) Magnetic-bead based method c) Organic method. These methods are discussed as follows:

DNA purification from FTA cards: The DNA purification from FTA cards was performed using the technique described by Sahajpal et al.[18]. In brief, the punching of FTA cards containing dried blood samples was done with a micro puncher. The punches were put into the micro vials (1.5ml) and mixed with purification reagent (150µl) and proteinase K (15µl) followed by incubation at 56°C for two hours in a water bath (NB 20 Nuve, Ankara, Turkey). The purified punches were then rinsed three times with autoclaved ultrapure water before being dried in a digital dry bath (Labnet International, USA). The punches were kept in a deep freezer at -20°C for later use.

Magnetic-bead based method: The DNA was extracted from blood samples on cotton gauze, blood samples, skin tissue, sternum, and ribs bones by magnetic bead-based method (Qiagen EZ1 Advanced XL BioRobot)[19]. A small piece of cotton gauze containing blood samples was cut with sterilized blades and placed into the vials (1.5 ml). The soft tissue of skin, sternum and ribs bones were chopped into pieces and placed into micro vials (1.5 ml). Approximately, 50 µl of liquid blood samples were added into the micro vials (1.5 ml). To all samples, buffer G2 (300 µl) and proteinase K (15 µl) were mixed followed by lysis at 56°C for 48 hours in a water bath. After lysis, each sample's lysate was poured separately into sample tubes (2.0 ml). The components of EZ1 DNA Investigator kit was loaded into BioRobot according to the manufacturer's instructions. The "Large-Volume Protocol" was selected for genomic DNA extraction and extracted DNA was stored in a deep freezer at 20°C for later usage.

Organic method: DNA from muscle tissue, hairs, teeth, and bones viz. finger, femur, jaw, hummers, clavicle, skull, radius, thumb, ulna, and tibia were extracted using the organic method[20-22]. A small portion of muscle tissue and hairs were cut into pieces and placed into the micro vials (1.5ml), respectively. The teeth samples were scraped using clean blades, put into falcon tubes and incubated overnight in absolute ethanol. After incubation, the ethanol was carefully drained followed by drying of teeth at room temperature. With the use of a hammer, the dried teeth were broken up into small bits, and the root and pieces were placed to micro vials (1.5ml). To eliminate microbial contamination, the bone samples i.e., finger, femur, jaw, humerus, clavicle, skull, radius, thumb, ulna and tibia were cleaned from the outer surface with sterilized blades. The bones were hammered into pieces and put in absolute ethanol for overnight. The ethanol was carefully drained out and bones were allowed to dry for 72 hours at room temperature. The dried bones were powdered using tissue lyzer (Qiagen, Germany). The DNA extraction buffer (350µl) and proteinase K (25µl) were put to the pieces of muscle tissue, hair, and teeth, mixed, and incubated at 56°C for 48 hours in a water bath. Besides this, powdered bones were placed in falcon tubes (15ml) and DNA extraction buffer (2ml), 2ml EDTA (0.5M) and proteinase K (25µl) were added. The falcon tubes were vortexes followed by incubation at 56°C for 72 hours in a water bath. The lysis product of each sample was placed into a separate vial (1.5 ml), to which 500µl of phenol, chloroform, and isoamyl alcohol (25:24:1) was added. These vials were then vortexed briefly and placed for ten minutes in a rotospin. The micro vials were then centrifuged for 10 minutes at 12,000 rpm in a refrigerated centrifuge. The aqueous layers containing DNA were carefully removed with a micropipette and poured in fresh vials. Then, 500µl of phenol, chloroform, and isoamyl alcohol was added followed by repetition of the preceding step once. The sodium acetate (2M) (100µl) and chilled absolute ethanol (1000µl) were poured to the aqueous layer. For overnight precipitation, the micro vials were placed in a refrigerator at -20°C (Celfrost, India). Then, vials were centrifuged at 14000 rpm for 15 minutes in a refrigerated centrifuge. The supernatant was carefully removed, followed by the addition of 70% ethanol (500µl). The tubes were centrifuged at 10000 rpm for five minutes and the supernatant was removed. After repeating the step once, vials containing genomic DNA were dried at 56°C for 30 minutes in a dry bath. To micro vials, TE buffer (20µl) was added and incubated at 56°C for 10 minutes in a dry bath. The extracted DNA was stored in a refrigerator at -20°C for further use and quantified using agarose gel electrophoresis (0.8%). For the PCR amplification, one nanogram of genomic DNA was used.

PCR Amplification: The PowerPlex® 21 System (Promega Corporation, U.S.A.) and GlobalFiler™ kits (Thermo Fisher Scientific Inc., U.S.A.) were used for PCR amplification of extracted and purified DNA. The PCR amplification was performed using GeneAmp® PCR 9700 and Veriti™ 96-Well thermal cyclers (Applied Biosystems, U.S.A.) as manufacturer's instructions.

Capillary electrophoresis and genotyping: The genotyping of PCR products from PowerPlex® 21 kit was done with ABI 3130 Genetic Analyzer (Applied Biosystems, U.S.A.) with GeneMapper® ID Software v 3.2. Besides this, capillary electrophoresis of amplified PCR products from GlobalFiler™ PCR kit was done with ABI 3500 and 3500 XL Genetic Analyzers (Applied Biosystems, U.S.A.) using GeneMapper™ ID‑X Software v 1.6.

Results and Discussion

The results of the current study depicted that the most of the unidentified dead bodies in Himachal Pradesh, India were in the age group of 21-40 years (38.2%) and 41-60 years (34.6%). However, the percentage of age groups between 0-20, 61-80, 81-100 years were 3.8%, 8.6%, and 0.4%, respectively. The age of few unidentified dead bodies (14.4%) could not be determined due to advanced stages of decomposition (Figure-1).

The gender distribution of unidentified dead bodies is depicted in Figure-2. Majority of them were males (91.8%) and only 8% were female. The gender of the remainder of the unidentified carcasses (0.2%) could not be determined due to the highly degraded nature of the samples, which did not yield any DNA profile. Majority of the unidentified deceased were found in a putrefied state (32%), while fresh and partially decomposed bodies were 30% and 26.8%, respectively. The dead bodies in the advanced stage of decomposition (5.8%), skeletonized (4.6%), and burnt (0.8%) conditions were also retrieved (Fig. 3). As shown in Fig. 4, the majority of unidentified dead bodies were recovered from the river (12.8%) and hospital (11.6%). The other sites were ground (7%), water course (6.6%), road (6%), village (6%), dam (4.4%), forest (3.2%), railway track (3%), house (3%), lake (2.8%), rain shelter (2.6%), market (2.4%), bridge (2.2%) and bus stand (2%). Figure-5 depicted season-wise detail of recovered unidentified dead bodies. Most of the unidentified dead bodies were reclaimed in the rainy season i.e., July to September (29.4%) and least from the winter season i.e., October to December (22.6%). Table-1 depicts the DNA profiles in percentage from various samples of unidentified deceased. As per Table-1, bones such as thumb, humerus, clavicle, radius, ulna, and skin tissue generated complete DNA profiles, whereas sternum bone, femur bone, ribs bone, blood sample on cotton gauze, teeth, finger, blood sample on FTA cards, blood, muscle tissue, hair and tibia bone showed 97%, 92%, 89%, 87.5%, 85%, 80%, 79%, 67%, 61%, 50% and 50% percent DNA profiles, respectively. The jaw bone, and skull bone showed no result.

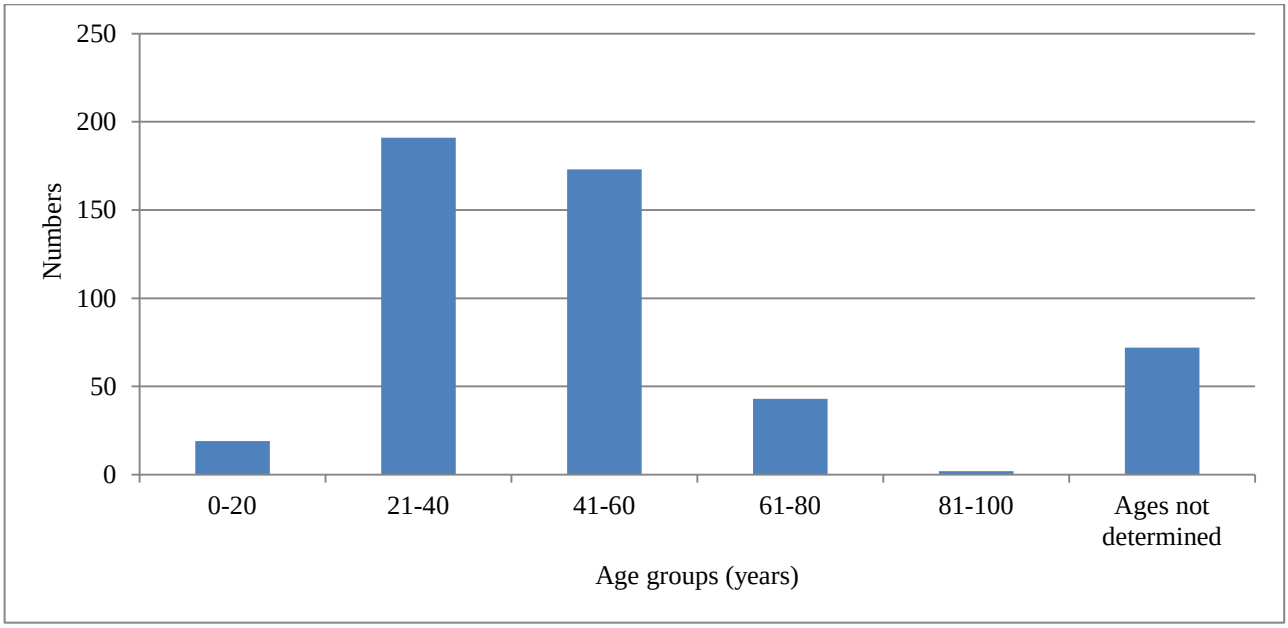

Figure-1: Distribution of unidentified dead bodies with age groups.

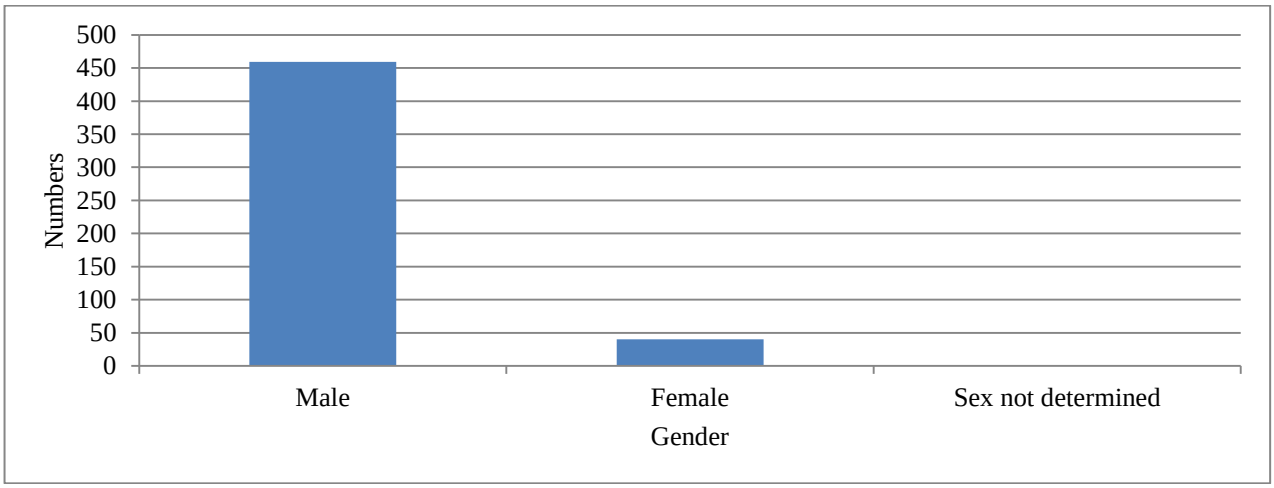

Figure-2: Gender-wise distribution of unidentified dead bodies.

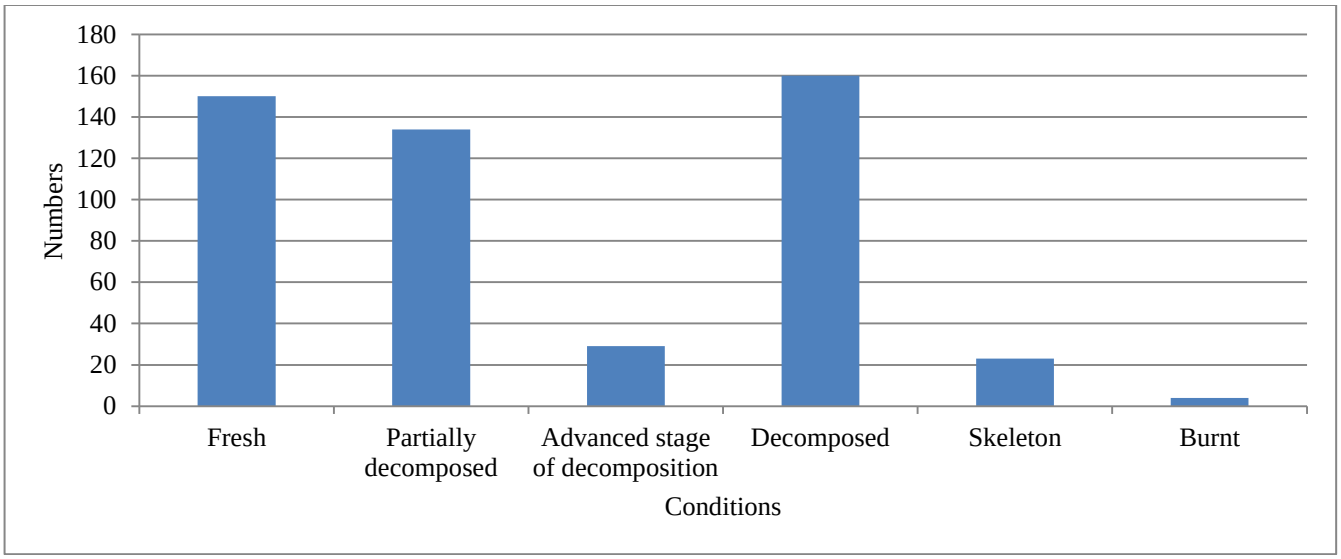

Figure-3: Conditions of recovered unidentified dead bodies.

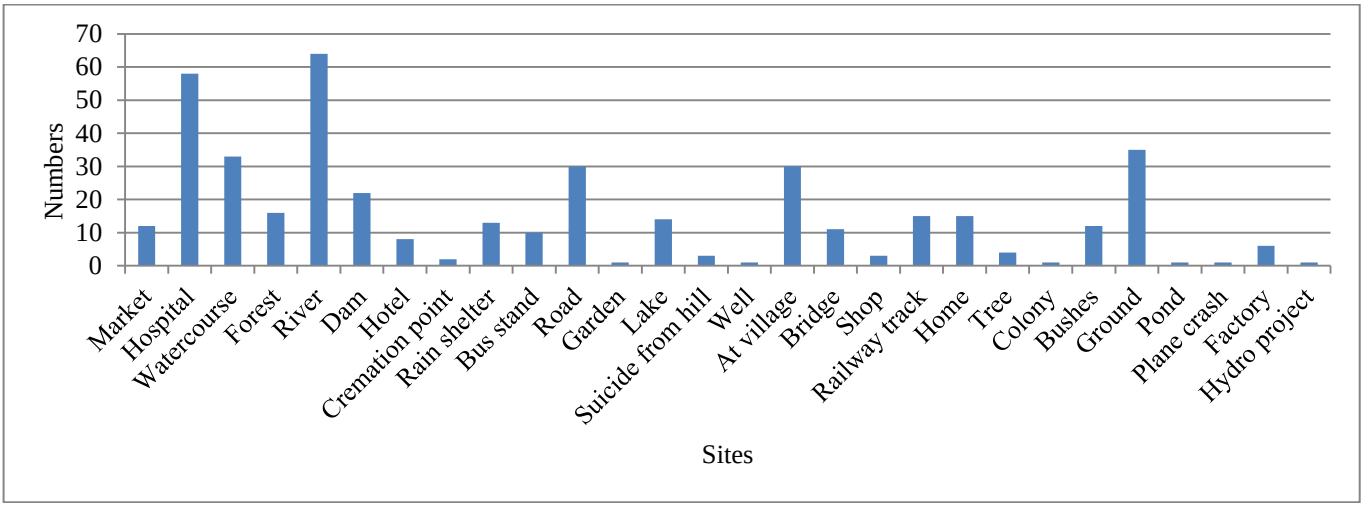

Figure-4: Sites of recovery of unidentified dead bodies

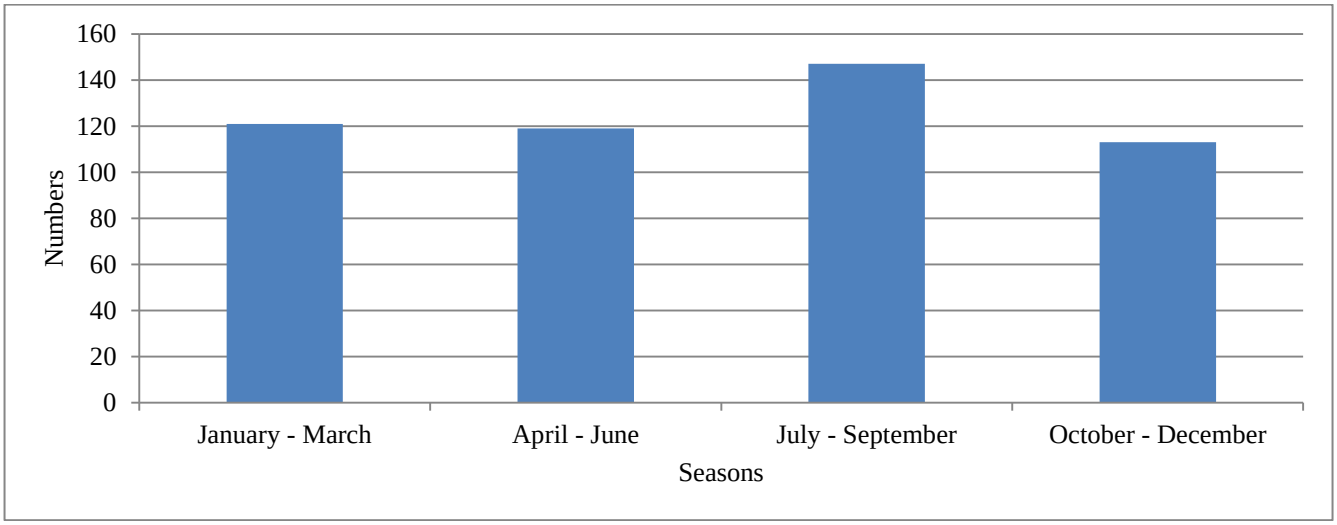

Figure-5: Season-wise details of recovered unidentified dead bodies.

Discussion: Demographic data of Himachal Pradesh showed that the most of unidentified dead bodies were in the age group of 21-40 years with majority being males. This may be because males of this age group often leave for work in other places and lose their lives due to accidents, drowning, suicide, etc. Similar to this study, Kumar et al.[23] conducted a retrospective study (2001-2005) in South Delhi and reported that the age group of unidentified homeless persons ranged from 31-40 years with 87.7% male individuals. In another study, Yadav et al.[24] conducted a five-year retrospective analysis (2010-2014) on 7964 cases referred for medico-legal autopsy at a hospital in New Delhi, India. They found that the unidentified and unclaimed dead bodies accounted for 16%. They also reported that males outnumbered females and half of the unidentified dead bodies were in the 31-50 age groups. Shandil et al.[25] did a prospective study (2016-2018) on 114 medico-legal autopsies at Patna Medical College, Bihar, and reported that 78.07% of unidentified dead bodies were males who belonged to the age group of 21–30 years (24.5%) followed by 41-50 years (23.68%). Chaudhary et al.[26] conducted a study on 749 cases (2006-2010) on deaths of homeless unknown persons in New Delhi and reported that the majority were males (90.25%) with the age group of 41-50 years, followed by 31-40 years (24.43%). Prasad et al.[27] conducted a study on 5103 autopsies at the Department of Forensic Medicine and Toxicology, Patna Medical College, Bihar, India between August 2012 to September 2014 and found that 401 (7.85%) were unidentified cadavers. They also reported that 342 unidentified dead bodies (85.28%) were male with the middle age group of 30-50 years (42.64%). Arora and Singh[28] conducted a prospective study on 144 unclaimed dead bodies from 1st April 2014 to 31st March 2015. They also reported that 138 (96%) were males and most victims (69%) were in the age group of 21 to 50 years.

In the current study, it was observed that the majority of the unidentified deceased were recovered in decomposed conditions as sometimes dead bodies remain undiscovered due to tough topography and terrain of Himachal Pradesh. The most of unidentified dead bodies were reclaimed from the river in the rainy season (July to September). This could be due to the massive floods and landslides triggered by heavy rains in Himachal Pradesh, which led to extensive damage. Kumar et al.[23] also reported a major number of dead bodies (32.2%) from the river or its bank in South Delhi during rainy season (July to September). Also, Chaudhary et al.[26] reported a maximum of 109 (14.5%) deaths in the month of September. In contrast to this study, Arora and Singh (2017) reported a maximum number of unclaimed deaths in the winter season (15%).

The genotypes were prepared from various samples of unidentified bodies using PowerPlex® 21 System and GlobalFiler™ kits and both kits were found to be of equally good quality. As shown in the Table-1, samples such as bones (thumb, humerus, clavicle, radius, ulna, sternum, femur, ribs, finger) and teeth, skin tissue, blood sample on cotton gauze showed satisfactory recovery of DNA profiles as DNA was better preserved in those samples that yielded good quality and sufficient amounts of DNA. However, samples like blood sample on FTA card, blood, muscle tissue, hair and tibia bone yielded fair quantity of the DNA resulted in complete and partial DNA profiles. In comparison, jaw bone and skull bone yielded degraded DNA in insufficient amounts and therefore no DNA profiles could be generated. These findings concluded that medical professionals should preferentially collect and send samples such as bones viz. thumb, humerus, clavicle, radius, ulna, sternum, femur, ribs, finger and skin tissue, teeth, blood sample on cotton gauze for DNA profiling of unidentified dead bodies to forensic science laboratories depending upon their conditions. Moreover, the samples such as blood sample on FTA card, blood, muscle tissue, hair and tibia bone can be forwarded if previous mentioned samples are not available for DNA profiling.

Conclusion

The unidentified dead bodies are recovered by the police from disasters such as mass accidents, forest fires, avalanches, armed conflicts, etc. These dead bodies are recovered either in fresh or decomposed conditions, which puts considerable pressure on law enforcement agencies. This study concluded that in addition to age, sex, conditions, locations and month of recovery of unidentified dead bodies, proper collection and preservation of exhibits can play an important role in generating DNA profiles and identifying the unknown deceased. Comparing the DNA profiles of an unknown deceased with worried relatives can provide answers to their relationship, which can help in obtaining death certificates, judicial releases, justice and a proper burial for the deceased. There is also a need for a database of DNA profiles for cross-checking between unidentified bodies and missing persons, which should be accessible to both law enforcement and civilians searching for their loved ones.

Acknowledgements

The authors are thankful to the Director, DFS, Junga, Shimlato provide the facilities to perform this work as a routine case work.

References

1. National Crime Records Bureau. (2015). Number of Unidentified Dead Bodies Recovered and Inquest Conducted During 2015.

2. Kumar, N., Chauhan, A., Gupta, R., Maitray, A., Sharma, D. and Shukla, S.K. (2019). Effect of fire on DNA and its profiling in homicide cases. *Forensic Res Criminol Int J.*, 7, 90-94.

3. Prinz, M., Carracedo, A., Mayr, W. R., Morling, N., Parsons, T. J., Sajantila, A., ... & Schneider, P. M. (2007). DNA Commission of the International Society for Forensic Genetics (ISFG): recommendations regarding the role of

forensic genetics for disaster victim identification (DVI). *Forensic Science International: Genetics*, 1(1), 3-12.

4. Alonso, A., Martin, P., Albarrán, C., Garcia, P., de Simon, L.F., Iturralde, M.J., Fernández-Rodriguez, A., Atienza, I., Capilla, J., García-Hirschfeld, J., Martinez, P., Vallejo, G., García, O., García, E., Real, P., Alvarez, D., León, A. and Sancho, M. (2005). Challenges of DNA profiling in mass disaster investigations. *Croat Med J*., 46, 540-548.

5. Budowle, B., Bieber, FR. and Eisenberg, A.J. (2005). Forensic aspects of mass disasters: Strategic considerations for DNA-based human identification. *Legal Med*., 7, 230-243.

6. Kumar, N., & Sharma, A. (2021). Human identification through DNA analysis of teeth using powder-free method–A case study. *The Journal of forensic odonto-stomatology*, 39(1), 45.

7. Roewer, L. (2013). DNA fingerprinting in forensics: past, present, future. *Investigative genetics*, 4, 1-10.

8. Taki, T., Machida, M., & Shimada, R. (2019). Trends of traffic fatalities and DNA analysis in traffic accident investigation. *IATSS research*, 43(2), 84-89.

9. Artés, T., Oom, D., De Rigo, D., Durrant, T. H., Maianti, P., Libertà, G., & San-Miguel-Ayanz, J. (2019). A global wildfire dataset for the analysis of fire regimes and fire behaviour. *Scientific data*, 6(1), 296.

10. Biesecker, L.G., Bailey-Wilson, J.E., Ballantyne, J., Baum, H., Bieber, F.R., Brenner, C., Budowle, B., Butler, J.M., Carmody, G., Conneally, P.M., Duceman, B., Eisenberg, A., Forman, L., Kidd, K.K., Leclair, B., Niezgoda, S., Parsons, T.J., Pugh, E., Shaler, R., Sherry, S.T., Sozer, A. and Walsh, A. (2005). DNA identifications after the 9/11 world trade center attack. *Sci*.,310, 1122-1123.

11. Nedić, D., & Pilija, V. (2020). The challenges of forensic medical expertise in aircraft accidents: A case report. *Journal of Indian Academy of Forensic Medicine*, 42(1), 63-65.

12. Marjanović, D., Metjahić, N.H., Čakar, J., Džehverović, M., Dogan, S., Ferić, E., Džijan, S., Škaro, V., Projić, P., Madžar, T., Rod, E. and Primorac, D. (2015). Identification of human remains from the second world war mass graves uncovered in Bosnia and Herzegovina. *Croat Med J.*, 56, 257-262.

13. Ziętkiewicz, E., Witt, M., Daca, P., Zebracka-Gala, J., Goniewicz, M., Jarzab, B. and Witt, M. (2012). Current genetic methodologies in the identification of disaster victims and in forensic analysis. *J Appl Genet*., 53, 41-60.

14. Saukko, P. and Knight, B. (2005). The establishment of identify of human remains. In: Saukko P (ed) Knight's Forensic Pathology. 3rd ed. Arnold: A member of the Hodder Headline Group, London, UK, pp 98-135.

15. Lynch, M. (2003). God's signature: DNA profiling, the new gold standard in forensic science. *Endeavour*., 27, 93-97.

16. Behera, C., Singh, P., Shukla, P., Bharti, D.R., Kaushik, R., Sharma, N., Mahapatra, BK., Chauhan, K., Meher, S.K., Yadav, A., Tabin, M., Gupta, S.K and Singh, H. (2022). Development of the first DNA database and identification portal for identification of unidentified bodies in India – UMID. *Sci Justice*., 62, 110-116.

17. Himachalpr Gov. (2003). H.P. at a Glance. http://himachalpr.gov.in/Index. aspx?Data=31.

18. Sahajpal, V., Rajput, S., Sharma, T., Sharma, A., & Thakar, M. K. (2019). Development and evaluation of a novel DNA purification buffer and protocol for blood samples on FTA cards. *Forensic Science International: Reports*, 1, 100014.

19. EZ1&2® DNA Investigator® Kit Handbook. https://www.qiagen.com/us/products/human-id-and-forensics/investigator-solutions/ez1-2-dna-investigator-kit.

20. Thatcher, S. A. (2015). DNA/RNA preparation for molecular detection. Clinical chemistry, 61(1), 89-99.

21. Mullegama, S. V., Alberti, M. O., Au, C., Li, Y., Toy, T., Tomasian, V., & Xian, R. R. (2019). Nucleic acid extraction from human biological samples. *Biobanking: Methods and protocols*, 359-383.

22. Kumar, N., Sharma, A., & Kumari, V. Establishment of paternity from humerus bone of an unidentified skeleton.

23. Kumar, A., Lalwani, S., Behera, C., Rautji, R., & Dogra, T. D. (2009). Deaths of homeless unclaimed persons in South Delhi (2001-2005): a retrospective review. *Medicine, Science and the Law*, 49(1), 46-50.

24. Yadav, A., Kumar, A., Swain, R. and Gupta, S.K. (2017). Five-year study of unidentified/unclaimed and unknown deaths brought for medicolegal autopsy at Premier Hospital in New Delhi, India. *Med Sci Law*., 57, 33-38.

25. Shandil, A., Prasad, M. and Choudhary, R. (2019). Pattern of death of unidentified cases: A mortuary based study from Patna Medical College, Bihar. *J. Med Sci Clin Res*., 07, 1004-1007.

26. Vidua, R. K. (2016). An investigation in to the deaths of homeless unknown persons (HUPs) at New Delhi District. *EC Pulmonology and Respiratory Medicine*, 2, 90-97.

27. Prasad, M., Kumar, A., Kumar, S., Ranjan, S. K., Goel, N., & Singh, A. K. (2019). Study of Medicolegal Aspects of Unknown Cases in PMCH, Patna.

28. Pankaj, A., & Singh, B. R. (2017). Dealing with unclaimed dead bodies-embrace the challenge. *International Journal of Community Medicine and Public Health*, 4(4), 992.