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## Original Article

**Mitochondrial DNA (8389-8865 base pairs) mutation search in Turkish population**Omer Karatas<sup>1</sup>, Havva Altuncul<sup>1</sup>, Nazli Holumen<sup>1</sup>, Itir Erkan<sup>2</sup>, Dilek Salkim Islek<sup>1</sup>, Emel Hulya Yukselglu<sup>1</sup><sup>1</sup>Istanbul University-Cerrahpasa, Institute of Forensic Sciences and Legal Medicine, Istanbul, Türkiye<sup>2</sup>Istanbul Yeni Yuzyl University, Faculty of Health Sciences, Department of Health Management, Istanbul, Türkiye

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**Abstract**

**Aim:** STR loci in nuclear DNA (nDNA) is generally used for identification and identity determination in forensic sciences. However, it is not always possible to achieve successful results from these loci. Especially in biological samples containing nDNA that are degraded in quantity and quality, there might be difficulties to analyze. In such cases, it may be more reliable to use mitochondrial DNA (mtDNA) analysis. Because there is only one nDNA in the cell, while there are many copies of mtDNA. Therefore, it is advantageous to work with. HVI, HVII and HVIII regions in mtDNA are used for identification purposes in forensic sciences. However, the discrimination power of these regions are limited. As an alternative to these regions, studying the coded region will increase the discrimination power. For this reason, the aim of our study is to distinguish the points showing polymorphism in the region between 8389-8865 base pairs as an alternative to the HVI, HVII and HVIII regions. In addition, it will be optimized for routine use in biological samples that do not contain enough nDNA with the regions where the polymorphism rate is determined.

**Materials and Methods:** In our study, blood samples were taken from 150 volunteers from the Turkish population who were not related. The region in question was reproduced in accordance with the method of PCR Tzen et al. The obtained sequences were compared with the Cambridge Reference Sequence.

**Results:** The most common polymorphisms were found to be 8860G, 8697A, 8847delC, 8691delA. The discrimination power of the studied region was calculated to be 0.923641, the probability of random matching was 0.076359 and the genetic diversity was calculated as 0.9303827.

**Conclusion:** In line with these data, it was determined that we could distinguish between individuals with the region we examined and that we could increase the discrimination power by analyzing this region together with the HVI, HVII, HVIII regions.

**Keywords:** Mitochondrial DNA, identification, forensic genetics, polymorphisms**INTRODUCTION**

Polymorphic STR loci in nDNA are commonly used for identification and lineage determination in forensic sciences. However, biological samples that come to the laboratory often contain degraded nDNA in terms of both quantity and quality, rendering traditional STR studies inadequate or unsuccessful. In such cases, mtDNA, which shows maternal inheritance, is used due to its hundreds of copies in each cell and high mutation rate. MtDNA is particularly advantageous when working with bone,

teeth, hair, and other materials with highly degraded samples [1]. Because of these features, mtDNA is utilized in forensic science to determine migration routes, in evolution studies, and also to research various diseases [2].

The D-loop region in mtDNA is commonly examined in forensic sciences, as it is polymorphic and does not encode any genes [3]. In identification and lineage studies, the HVI and HVII segments of this region are typically analyzed to obtain information. However, this information is limited and has low discrimination power [4].

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To increase the power of discrimination, researchers investigate the coding regions of mtDNA, which have a lower number of bases and may have links to diseases [5].

Population studies are crucial in forensic sciences to determine the distribution of alleles or genotypes in a given population. This information is essential for forensic identification and object determination. However, in the Turkish population, there is a lack of mutation and single nucleotide polymorphism (SNP) information in the region between bases 8389-8865 of mtDNA. This region may have potential forensic value, but without information on the population's variation, it cannot be utilized effectively. Therefore, to benefit from this region, it is necessary to conduct a population study to determine the distribution of mtDNA mutations and SNPs in Turkey. It is important to note that mutation rates can vary significantly among different populations, and it is imperative to identify mutations specific to the Turkish population to ensure accurate forensic identification. The aim of this study is to sequence the mtDNA coding region between base pairs 8389-8865 and identify any mutations specific to the Turkish population. This will provide valuable information for forensic scientists in population identification and object determination. By utilizing mtDNA polymorphism, forensic scientists can determine whether the Turkish population's mutation profile is favorable for forensic identification purposes. Conducting population studies in this area is essential for accurate forensic identification, and identifying mutations specific to the Turkish population is crucial in utilizing this region effectively.

## MATERIAL AND METHOD

One hundred and fifty individuals aged over 18 years, who had no consanguineous relationship, and had given their informed consent, were randomly selected to participate in this study. Blood samples were collected from the participants and stored at -20°C until the analysis. The study was conducted with the approval of the Istanbul University Cerrahpasa Medical Faculty Clinical Research Ethics Committee (permission dated 02.06.2011, No. 19245), and all participants signed an informed consent.

The DNA isolation from the collected blood samples was performed using the Invitrogen Purelink Genomic DNA Mini Kit® (Thermo Fisher Scientific) which is based on silica extraction technology. DNA amounts of the obtained isolates were determined by fluorometric method with Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific). Tzen et al. (2001) method was modified and samples were multiplied using TT20173-F (5'-CCCCTCTAGAGCCCATAAAGC-3') and TT29174-R (5'-GTGCATGAGTAGGTGGCCTGC-3') primers in the first phase of PCR amplification. For the PCR reaction, 5.0 µL 1X PCR buffer, 2.0 µL 1.5 mM MgCl<sub>2</sub>, 0.2 µL 1U AmpliTaq Gold DNA polymerase (Applied Biosystems), 4.0 µL 0.125 mM dNTP mixture, and 1.0 µL 1 µM for each primer were used. DNA samples were diluted to 1-2 ng and added to the PCR mixture. It was completed with distilled water with a total volume of 50 µL. PCR was performed on 9700 (Applied Biosystems) device. PCR

cycle; denaturation at 95°C was set to 32 cycles and 10 minutes at 72°C for 10 minutes denaturation, 1 minute at 94°C, 1 minute at 56°C, and 1 minute at 72°C. PCR products include primers and dNTPs that are not bound outside the target region. Escherichia coli Exonuclease I (Exo I) and Shrimp Alkaline Phosphatase (SAP-Shrimp Alkaline Phosphatase) were used to remove non-reactive dNTP and primers after PCR. For this process, 0.65 µL (1U/µL) SAP and 0.35 µL (5U/µL) Exo I were added on each 3.75 µL PCR product. The mixture was heated at 37°C for 90 minutes and at 80°C for 20 minutes.

BigDye Terminator v3.1 Cycle Sequencing Kit was used in the second phase of PCR amplification (Thermo Fisher Scientific). The second PCR mixture was prepared on a cold mold in order not to be affected by heat. For the PCR reaction, a mixture containing 4.0 µL BigDye Terminator v3.1 ready reaction mixture, 2.0 µL 5X sequencing buffer and 1.5 µL sequencing primer per sample was prepared. 1 µL of purified PCR product was added for the sample to this mixture. As a positive control, 1 µL -M13 control primer and pGEM®-3Zf (+) double chain control DNA and 1 µL distilled water were used as negative control. The total volume was completed with 20 µL of distilled water. PCR cycle; denaturation was set at room temperature for 10 seconds at 96°C, 5 seconds at 50°C, and 4 minutes at 60°C. Zymogen Sequencing Clean Up Kit was used to remove unbound fluorescently labeled ddNTPs in the environment for sequencing of the second PCR products (Zymo Research).

Electrophoresis of PCR products was performed on the ABI Prism 310 Genetic Analyzer (Applied Biosystems) using Data Collection Software 3.0. Data analysis was done with GeneScan Analysis Software 3.1.2 (Applied Biosystems), Seqscape, Finch TV, Sequencher 4.10.1. Polymorphic points were determined by comparing mtDNA profiles obtained from samples with Cambridge Reference sequence preloaded in SeqScape program.

## RESULTS

To assess the repeatability of the method, five samples were randomly selected and re-analyzed by the same person at different times. The results showed that the same genotype was obtained each time, indicating high repeatability. To determine the polymorphic points, all samples were compared with the Cambridge Reference Sequence. The analysis revealed that the most common mutation detected in the samples was 8860a> G, (found in 88 individuals). Other mutations included 8697g> A (found in 19 individuals), 8847delC (found in 8 individuals), and 8691delA (found in 13 individuals). Additionally, 12 individuals had the same genotype as the Cambridge Reference Sequence. Table 1 presents the common mutation results and comparisons with German and two different populations of Taiwan for the Turkish population [4-7]. Haplotypes were grouped according to the polymorphisms detected in our study. People with the same genotype as the Cambridge Reference Sequence were not included in this grouping. Discrimination power and random match probabilities

values were calculated.

**Table 1.** Comparison of mtDNA with German and Taiwanese population in terms of mutation rates

Position	Number of persons (n=150)	Turkish population (%)	German (%)	Taiwan (%)	Taiwan (%)
8860a>G	88	58.6	64	61.2	51.2
8697g>A	19	12.6	6.1	-	-
8691delA	13	8.6	-	-	-
8847delC	8	6	-	-	-
8679delA	6	4.34	-	-	-
8828delA	5	3.62	-	-	-
8481c>T	5	3.62	-	-	-
8815delC	5	3.62	-	-	-
8817delA	5	3.62	-	-	-
8691a>C	4	2.89	-	-	-
8701a>G	3	2.17	-	52.5	39.5
8354c>T	-	-	3.3	-	-
8448t>C	-	-	2.8	-	-
8473t>C	1	0.72	2.8	-	-
8584g>A	1	0.72	-	-	14.3
8414c>T	1	0.72	-	-	8.4

The most common haplotypes are 8860a> G (36 individuals), 8697g> A (7 individuals), 8860a> G (7 individuals), 8847delC (3 individuals), 8691delA (2 individuals), 8512–8513insA (2 individuals) and 8697g> A (3 individuals). In our study, a total of 38 insertion and 95 deletion-type changes were seen, and the base changes resulting from transversion are shown in Table 2.

**Table 2.** Details of the mutations observed in mtDNA

Mutation Type Transition		Mutation Type Transition	
Transition	Number	Transversion	Number
t>C	13	a>C	16
c>T	27	c>A	9
g>A	29	a>T	7
a>G	100	t>A	13
<b>TOTAL</b>	<b>169</b>	g>T	0
		t>G	4
<b>Mutation Type</b>	<b>Number</b>	g>C	1
<b>Insertion</b>	38	c>G	7
<b>Deletion</b>	95	<b>TOTAL</b>	<b>57</b>

The following values were obtained as a result of random match probability, discrimination power and genetic diversity calculation.

Xi: Haplotype number, n: Number of persons

Xi: It was calculated by dividing the number of people seen by the total person.

**Probability of random matches;** $P = \sum (Xi^2)$

0.076359

**Discrimination power;** $DP = 1 - \sum (Xi^2)$

=0.923641

**Genetic diversity;**

$$h = \frac{(1 - \sum X^2)n}{(n-1)}$$

It was calculated as=0.9303827.

**DISCUSSION**

mtDNA analysis is a routinely used forensic technique that helps resolve many cases. Alternative methods, such as mtDNA sequence analysis, are used when routine analysis methods fail. When using mtDNA sequence analysis, assessments are typically made based on the polymorphism results in the HVI and HVII regions of mtDNA, but these regions have limited discriminatory power. Eight polymorphisms in the mtDNA coding region (8281–8289d, 1736, 13263, 4883, 3594, 10873, 10400, and 12705) have been studied in other regions of the world using phylogenetic analysis of the HVI and HVII regions to identify strains of different origins [8]. This study aims to identify mutations in the Turkish population by performing sequence analysis of the mtDNA coding region between base pairs 8389-8865. We suggest that this polymorphic region can increase the discriminatory power of forensic identification studies of mtDNA in the Turkish population.

The selected region has little effect on the phenotype and has been excluded from the study if associated with disease, such as point myoclonic epilepsy with ragged-red fiber (A → G(8344)), ataxia (G → A(8363)), neurogenic muscle weakness (T → G(8993)) and retinitis pigmentosa (T → C(8356)), due to ethical considerations in forensic science research [6, 9-11].

Andrews et al. re-analyzed the Cambridge Reference Sequence and compared it to the original sequence. They found that the a > G base change at position 8860 is a very common polymorphism worldwide [12, 13]. In our study, the most common mutation was the a> G change at position 8860, with a frequency of 58.6%. This mutation was found to be present in 64% of the German population, 61.2% in one of two different studies of the Taiwanese population and 51.2% in the other [4-7].

The 8697g> A mutation is the second most common mutation with a frequency of 12.6%. In the German population, this mutation has a frequency of 6.1%. Other common polymorphisms include 8691delA 8.6%, 8847delC 6%, 8679delA 4.34%, 8828delA 3.62%, 8481c> T 3.48%, 8815delC 3.62%, 8817delA 3.62%, 8691a> C 2.89%, 8701a> G 2.17%, and 8473t> C, 8584g> A, 8414c> T 0.72% each.

The 8701a> G mutation was found to have a frequency of 52.5% in one study in Taiwan and 39.5% in another. This ratio is significantly higher than the Turkish population (2.17%). Similarly, the frequency of the 8473t> C mutation in the German population was much higher than the Turkish population, with rate of 2.8%. The frequency of the 8584g> C, and 8414c> T mutations in the Taiwan population was much higher than the Turkish population, with rates of 14.3%, and 8.4%, respectively.

Furthermore, while the 8691delA, 8847delC, 8679delA, 8828delA,

8481c> T, 8815delC, 8817delA, and 8691a> C mutations were observed in the Turkish population, they were not found in the German and Taiwanese populations. On the other hand, the 8354c> T and 8448t> mutations were found to have rates of 3.3% and 2.8%, respectively, in the German population, but were not observed in the Turkish and Taiwanese populations.

In summary, the frequency of mutations varies among different populations. Therefore, it is important to study the prevalence of genetic mutations in specific populations in order to better understand the genetic diversity of these populations.

In this study, a total of 169 transition type mutations, 57 transversion type mutations, 38 insertion type mutations, and 95 deletion type mutations were identified. Notably, no transition type mutations showing a g>T change were found in this study. These findings suggest that there is significant diversity in terms of mutation types present in the Turkish population.

While analyzing mtDNA, researchers have discovered instances of heteroplasmy. Although the current study did not detect any heteroplasmy, it's important to note that different tissues from the same individual may exhibit heteroplasmy when analyzed [14]. As such, it's crucial to take heteroplasmy into account when comparing two DNA samples for forensic identification purposes. However, evaluating DNA samples in cases of heteroplasmy can be challenging. Based on our analysis, the probability of two unrelated individuals in the Turkish population having the same genotype in the region we studied was calculated to be 0.076359. This low ratio suggests that the likelihood of match chance is low. However, the discrimination power of the region we examined alone is not sufficient for forensic purposes and has been calculated to be 0.923641. To increase the discrimination power, it is necessary to evaluate polymorphic regions such as HVI and HVII in the mtDNA, expanding the analysis to include 9021 bases unrelated to diseases. A population study in the United States showed that mtDNA examination increased the discrimination power for the US Caucasus and US Hispanic populations. To further improve discrimination power, we suggest examining regions coded in individuals with similar HVI and HVII regions, as well as exploring highly informative polymorphic clusters in published or unpublished sequence data [15]. In a recent population study conducted in the Liaoning region of China, 317 unrelated individuals were analyzed using mtDNA HVI, HVII, and coded regions. The study revealed that the Liaoning Han population is distantly related to the Tibet group but relatively close to the Miao group. The gene diversity (0.9997±0.0003), polymorphism information content (0.99668), and probability of random matching (0.00332) were also highly informative [16].

Other studies have shown that mtDNA analysis can yield successful results in both population studies and ancient bone samples. For instance, SNP points and control region HVI determined in the coding region, in combination with HVII regions, were used to analyze small samples from Joseon kingdom tombs in Korea dating back to the 1300s [17].

## CONCLUSION

Our study and other research highlight the importance of mtDNA analysis in identifying similarities and differences between populations when nDNA is insufficient, such as in determining lineage in biological materials. In future studies of Turkish populations, increasing the sample size and collecting samples from different regions can yield even more valuable insights.

### Conflict of interests

*The authors declare that there is no conflict of interest in the study.*

### Financial Disclosure

*The authors declare that they have received no financial support for the study.*

### Ethical approval

*The study was conducted with the approval of the Istanbul University Cerrahpasa Medical Faculty Clinical Research Ethics Committee (permission dated 02.06.2011, No. 19245), and all participants signed an informed consent.*

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Original Article

## Comparison of conventional heaney technique and ten step vaginal hysterectomy technique

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### Abstract

**Aim:** This study aimed to compare the conventional Heaney technique and the ten-step vaginal hysterectomy technique.

**Materials and Methods:** This retrospective study included 65 women who were operated vaginal hysterectomies at Kanuni Sultan Suleyman Training and Research Hospital between 1 October 2020 and 31 December 2021. Female patients aged between 40 and 85 who underwent vaginal hysterectomy for benign indications were enclosed in the study.

They were divided into two groups, Conventional Heaney's technique(CHVH Group 1) and the ten-step vaginal hysterectomy technique (TSVH Group 2). In the CHVH group, 39 were potentially eligible and 35 were included in the study. On the other hand, in the TSVH group, 33 were potentially eligible and 30 were included in the study.

**Results:** There were 8(22.85%) complications in the CHVH group and 4(13.33%) in the TSVH group. With the CHVH method, there were 2(5.71%) bladder perforations, 1(2.85%) converted to laparotomy, and 1(2.85%) laparotomy problem. In the TSVH approach, there was no bladder perforation, and no one converted to laparotomy. The group patients' operating times were longer in the CHVH method than in the TSVH technique, 56.6018.44 and 42.2018.63, respectively. Prolapsus uteri were the most common indication for surgery in both groups.

**Conclusion:** TSVH is superior to CHVH in terms of complication, and operating time, The ten-step vaginal hysterectomy technique can be used because of its low complication rates.

**Keywords:** Comparison, complication, heaney technique, ten-steps technique, vaginal hysterectomy

### INTRODUCTION

The most common operation performed on women in gynecology is a hysterectomy. Vaginal hysterectomy was first mentioned by Soranus of Ephesus. The first successful vaginal hysterectomy was done by Recamier in 1829. In 1853, Ellis Burnham reported the first successful abdominal hysterectomy [1]. In 1989, Reich et al. [2] performed the first laparoscopic hysterectomy When compared to abdominal or laparoscopic procedures, vaginal

hysterectomy has significant advantages. It can be done under epidural anesthesia, which is especially important for elderly women who are often in high-risk groups. Despite its benefits, vaginal hysterectomy is gradually losing its dominance in the surgical repertoire and is becoming phased out in many centers [3]. According to Driessen et al. [4], the number of laparoscopic hysterectomy procedures performed in the Netherlands grew from 3% in 2002 to 10% in 2007 and 36% in 2012. According to

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the findings of a 2015 Cochrane review[5], vaginal hysterectomy was superior to abdominal and laparoscopic techniques and recommended as the first-choice procedure for benign reasons

Over the years, so many vaginal hysterectomy techniques have been developed and practiced, such as the Porges, Falk, von Theobald, Heaney, Chicago, and Joel-Cohen methods. A standardized and simplified vaginal hysterectomy technique was required to evaluate complications in surgical approaches performed in the same or different centers. Michael Stark improved and pioneered the "Ten-Step Vaginal Hysterectomy" by re-evaluating six techniques [6].

The goal of this study was to compare the conventional Heaney technique and the ten-step vaginal hysterectomy technique.

## MATERIAL AND METHOD

This study retrospectively analyzed 65 female patients between the ages of 40 and 85 who were operated on for vaginal hysterectomy at Kanuni Sultan Suleyman Research and Training Hospital between October 2020 and December 2021. They were divided into two groups, ( Conventional Heaney technique, CHVH, and ten steps vaginal hysterectomy (TSVH). In the CHVH group, 39 were potentially eligible and 35 were included in the study. On the other hand, in the TSVH group, 33 were potentially eligible and 30 were included in the study.

All cases were classified according to the operative technique. Demographic and clinical features were compared, such as obstetric history, previous abdominal surgery, pelvic sonography findings, complications, treatment options, and length of hospital stay.

### Inclusion and exclusion criteria

Female patients between the ages of 40 and 85 who had a vaginal hysterectomy for benign reasons were included in the study. Patients with missing file information were excluded from the study.

The night before the surgery, the patients underwent mechanical colon cleansing with a rectal enema. Cefazolin sodium a dose of one gram was administered intravenously to all patients one hour preoperatively and six hours postoperatively. For thromboembolism prophylaxis, enoxaparin at a dose of 0.4 ml was administered subcutaneously 8 hours before the procedure and continued at 24-hour intervals throughout the hospitalization.

### Surgical technique

The CHVH technique began with an incision around the vaginal wall and cervix, then separated the bladder from the uterus, opened the anterior peritoneum, opened the posterior peritoneum, cut and ligated the uterosacral ligaments, cut and ligated the cardinal ligaments, ligated uterine vessels, delivered the uterine fundus outward, cut and ligated the tubo-ovarian and round ligaments, removed the surgical specimen. The round ligament and uterosacral-cardinal peduncles were fixated to the vaginal mucosa, reperitonized, and closed the vaginal mucosa [7].

The TSVH technique began with an incision around the vaginal

wall and cervix, then separated the bladder from the uterus, opened the posterior peritoneum, cut and ligated the uterosacral and cardinal ligaments together, ligated the uterine vessels, delivered the uterine fundus outward, opened the anterior peritoneum, cut and ligated the tubo-ovarian and round ligaments, removed the surgical specimen, left the peritoneum open, both sacro-uterine ligaments and paracervical tissues are connected for cul-de-sac obliteration repaired the pelvic floor, closed the vaginal wall [8].

**Ethical Approval:** This study conforms to the provisions of the Declaration of Helsinki and was approved by the ethics committee of Istanbul Kanuni Sultan Süleyman Training and Research Hospital KAEK/2022.09.199. Due to the study's retrospective character, the need for patient consent for both participation and publishing was waived. All patients provided written, fully informed consent before surgery. All the operations are performed by the same surgeon.

(SPSS Statistics for Windows Version 24.0 was used. The parameters distributed with normal distribution t-test was used. Independent t-test was used to compare paired groups, paired t-test was used to evaluate preoperative and postoperative variables, The parameters distributed with normal distribution explained by Mean+SD, Mann-Whitney U test was used for non-normally distributed parameters, The parameters distributed with non-normal distribution explained by Median(min-max) or median(25th-75thpercentiles). Chi-square and Fisher's exact test were used for comparisons of categorical variables. Significance and  $p < 0.05$  were evaluated.

## RESULTS

A total of 72 patients who underwent CHVH and TSVH surgeries in the obstetrics of a tertiary hospital between 01.10.2020 and 31.12.2021 were started in this retrospective research. Seven patients were eliminated from the trial because they were unable to complete the study or because their file information was missing. It was conducted on 65 patients. There were 4 non-participants in the CHVH and 3 in the TSVH so the CHVH technique was performed in 35 patients and the TSVH technique in 30 patients. The characteristics of the cases are shown in Table 1.

**Table 1.** Characteristics of patients

	CHVH	TSVH	p-value
Age, years (mean±SD)	56.32±8.85	55.18±0.7	0.45
Parity, n (mean±SD)	3.84±2.09	3.74±2.06	0.99
BMI, kg/m <sup>2</sup> (mean±SD)	26.04±2.11	25.96±2.16	0.13
<b>Menopausal status</b>			
Postmenopausal, n (%)	27 (30.0%)	24 (56.0%)	0.34
Premenopausal, n (%)	8 (70%)	6 (44.0%)	
<b>Previous surgery, n (%)</b>			
Simultaneous surgery (%)	8 (14.3%)	7 (14.0%)	0.47

SD: Standard deviation n: number, %: percentage  
CHV: Conventional Heaney vaginal hysterectomy. TSVH, Ten steps vaginal hysterectomy. An Independent t-test was used to compare two groups of continuous data. The Chi-Square test was used to compare two groups of categorical data. \*Statistically significant,  $p < 0.05$

There was no statistically significant difference in terms of mean age, mean parity, and body mass index, outcomes of vaginal hysterectomy techniques are given in Table 2.

**Table 2.** Operation time, hospital stay, and complications

	CHVH	TSVH	
	n (%)	n (%)	p
Bladder perforation	2 (%5.71)	0 (0)	0.02*
Bleeding requiring blood transfusion	1 (%2.85)	1 (%3.33)	0.62
Conversion to laparotomy	1 (%2.85)	0 (%0)	0.01*
Vaginal cuff infection	1 (%2.85)	0 (%0)	0.02*
Vaginal cuff dehiscence	1 (%2.85)	1 (%4)	0.03*
Vaginal cuff bleeding	1 (%2.85)	1 (%2)	0.46
Reoperation	1 (%2.85)	0 (%0)	0.02*
Pre-operative Hb(g/dl)	11.33±1.24	11.40±1.31	0.51
Postoperative Hb(g/dl)	9.60±0.74	9.53±1.44	0.43
Operation time (min)	56.60±18.44	42.2±18.63	0.01*
Hospital stay (d)	2.2±1.3	2.3±1.3	

min: minute, d: day, NS: not significant, n: number, %: percentage

\* Significant difference by 0.05 level

There were 8 complications (22.85%) in the CHVH group and 4 (13.33%) in the TSVH group. With the CHVH technique, there were 2 (5.71%) bladder perforations, 1 (2.85%) converted to laparotomy, and 1 (2.85%) relaparotomy problem. In the TSVH group, there was no bladder perforation, no relaparotomy, and no one was converted to laparotomy. The group patients' operating times were longer in the CHVH method than in the TSVH technique, 56.60±18.44 and 42.20±18.63, (p=0.01) respectively). There was no significant difference between the CHVH and TSVH groups in terms of length of hospital stay 2.2±1.4 and 2.3±1.1 days respectively. (p=0.32). There was a substantial difference between the CHVH and TSVH groups in terms of bladder perforation and operation times.

**DISCUSSION**

There were 8 complications (22.85%) in the CHVH group and 4 (13.33%) in the TSVH group. With the CHVH technique, there were 2 (5.71%) bladder perforations, 1 (2.85%) converted to laparotomy, and 1 (2.85%) relaparotomy. In the TSVH group, there was no bladder perforation, no relaparotomy, and no one was converted to laparotomy. The group patients' operation times were longer in the CHVH method than in the TSVH technique, 56.60±18.44 and 42.20±18.63, (p=0.01) respectively.

TSVH is superior to CHVH in terms of bladder complication, and operative time, The ten-step vaginal hysterectomy technique is more straightforward and more applicable and has better clinical results than the classical technique.

When the steps of both vaginal hysterectomy techniques are compared, some important differences are seen.

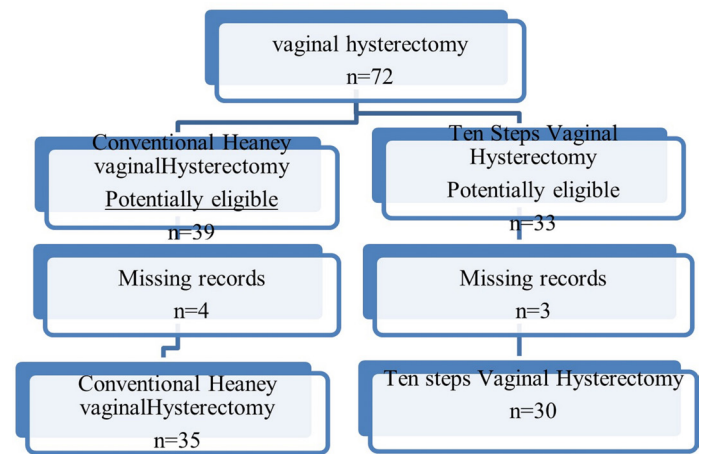
**a-**In the ten-step technique, an incision is made around the vaginal

wall and cervix first, and the anterior peritoneum is not opened immediately. In the Heaney technique, the anterior peritoneum is first opened to the vaginal mucosa.

**b-**In the ten-step technique, the Sacro-uterine ligament and paracervical tissues are held and tied with a single maneuver. In the Heaney technique, both sacro-uterine ligaments and paracervical tissues are cut in separate steps.

**c-**The peritoneum is left open in the ten-step technique. In the Heaney technique, the peritoneum is closed.

**d-**In the Ten step technique, both sacro-uterine ligaments and paracervical tissues are connected for cul-de-sac obliteration. In the Heaney technique, it does not bind.



**Figure 1.** Flowchart of vaginal hysterectomy

**Interpretation**

In CHVH trying to open the anterior peritoneum first leads and increases the risk of the urinary tract complication, In the Ten step technique binding Sacro uterine ligaments and cardinal ligaments together reduces the operation time. Leaving the peritoneum open allows the mobilization of intra-abdominal organs and fewer adhesions occur. In the Ten step technique, sacro-uterine ligaments and paracervical tissues are connected for cul-de-sac obliteration and to prevent internal organ diseases.

Bina I, et al. [9]. Reported that in ten steps technique compared with other techniques, there was a short hospital stay and less blood loss. Gorkem U, et al. [10] found shorter hospital stays, less operation time, and less analgesic requirement Zoricic D, et al. [11] reported shorter.

The studies comparing the TSVH and laparoscopic hysterectomy showed that despite the wide use of laparoscopy Ten steps vaginal hysterectomy should be a particular place in gynecology practice [12,13].

**Strengths and Limitations of the Study**

The data were meticulously collected, and the sample size was sufficient in comparison with the studies in the literature. This study determined the consequences of experience that could be

beneficial to each group. It can not be used for malignancies, severe adhesions, or large uterus. The study was retrospective and was conducted in a single Turkish tertiary care hospital. Those limitations may limit the ability to establish causal relationships and the generalizability of the study, hospital stay, less operation time, and less analgesic requirements. In this study, we didn't find any difference in terms of blood loss but found a significantly shorter operation time.

## CONCLUSION

The ten-step vaginal hysterectomy technique is more straightforward and more applicable and has better clinical results than the classical technique. TSVH is superior to CHVH in terms of complication, and operating time. The ten-step vaginal hysterectomy technique can be used because of its low complication rates. Clinical studies with larger samples are required to determine the generalizability of the study.

### Conflict of interests

*The authors declare that there is no conflict of interest in the study.*

### Financial Disclosure

*The authors declare that they have received no financial support for the study.*

### Ethical approval

*Approval was obtained from the Istanbul Medical Sciences Kamuni Sultan Süleyman Research and Training Hospital Ethics Committee Number:2022.09.199.*

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## Case Report

# Larynx and thyroid cartilage fracture after work accident and forensic medical evaluation

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## Abstract

Laryngeal injuries are rare but can be fatal. Depending on the type and severity of the trauma, thyroid and laryngeal cartilage fractures, full-thickness mucosal tears, tracheolaryngeal separation, and injuries leading to pneumomediastinum may be observed. Laryngeal traumas can occur either bluntly or penetratingly. Blunt traumas typically result from traffic accidents, falls, and sports injuries, while penetrating injuries are often caused by gunshot wounds or sharp objects. In this particular case study, the case was a 24-year-old male who was brought to the emergency room after being struck by a forklift at In his physical examination, a 10x1 cm wound was observed on his right scapula, along with subcutaneous emphysema in the subclavicular region. As a result of the examinations, the patient was found to have fractures of the larynx, thyroid cartilage, 8th rib and pneumomediastinum. In this study, it was thought that the thyroid and larynx cartilage fractures occurred as a result of hyperflexion/hyperextension of the neck without a direct blow to the neck region. This case is being presented because it is a rare occurrence in the literature, primarily due to its unique mechanism of injury.

**Keywords:** Occupational accident, tracheolaryngeal injury, forensic medicine

## INTRODUCTION

Laryngeal injuries are rare but can be fatal. It can be seen as a result of blunt trauma, but also as a result of sharp-penetrating tool and firearm injuries. It can also occur as a result of relatively minor damage to the anterior neck that causes posterior compression of the larynx against the spine [1-3].

Mortality rates due to larynx fractures have been reported to be up to 80% in non-hospitalized cases, while it has been reported to be 17.9% in hospitalized cases [4,5]. When a safe airway is provided and laryngeal injury is diagnosed correctly, the mortality

rate drops below 5% [6]. Early diagnosis and treatment have also been associated with improved voice, airway and swallowing outcomes [5-8].

As a result of blunt traumas, they can cause fractures, dislocations and thyroid cartilage and cricoid fractures in the laryngotracheal skeletal system with direct effect. In addition, they can cause such effects with their indirect effects in movements such as hyperflexion/hyperextension of the neck that will occur during trauma [1,2,8]. In this study, a case of tracheolaryngeal injury as a result of hyperflexion/hyperextension with the rare indirect effect of blunt trauma is presented.

## CITATION

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## CASE

Our case was a 24-year-old male. While working at work, he was brought to the emergency room after a forklift hit his back. In the first examination, it was seen that the patient was conscious, oriented and cooperative, blood pressure was 100/60 mm Hg, and pulse was 90/min. Physical examination revealed 10x1 cm ecchymosis and abrasion on the right scapula, and subcutaneous emphysema in the subclavicular region. There was no direct external examination characteristic for trauma in the neck region. In his systemic examinations, it was found that he had difficulty in breathing and hoarseness. Oxygen saturation level was determined as 98% with 10 L/min oxygenation. In his computed tomography, larynx and thyroid cartilage (figure 1 and figure 2) fractures were found together with pneumomediastinum (figure 1).

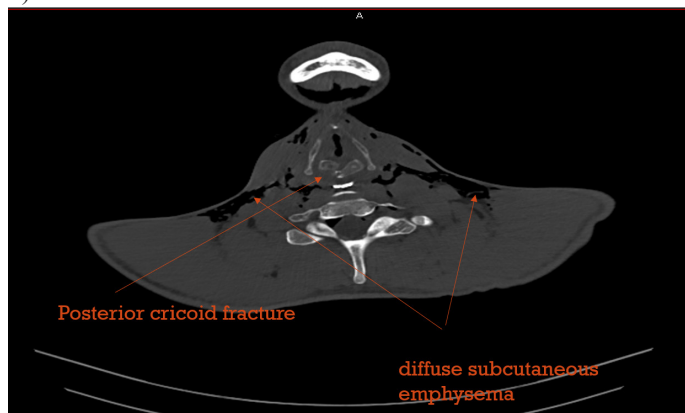


Figure 1. Posterior cricoid fracture and diffuse subcutaneous emphysema

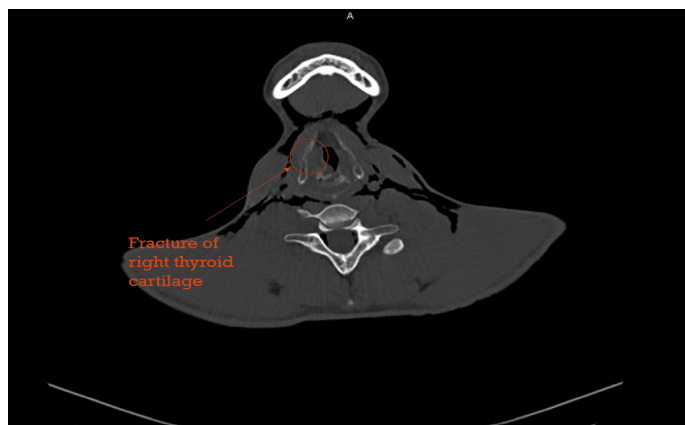


Figure 2. Fracture of right thyroid cartilage

According to the results of the radiographs evaluated as a result of the Thoracic and Cardiovascular Surgery consultation requested due to difficulty in breathing, a fracture was detected in the right 8th rib. And no hemothorax or pneumothorax was detected in the lungs. Fiberoptic laryngoscopy revealed a fracture of the right posterior cricoid cartilage, swelling in the vocal cords and mild bleeding. In the follow-up examination, it was observed that the swelling and bleeding in the vocal cords regressed. Therefore, tracheostomy was not opened. The patient, whose hoarseness and dyspnea regressed on the 8th day of his treatment, was

discharged at the end of the 10th day. In the follow-ups in the otolaryngology outpatient clinic, it was determined that the vocal cords and hoarseness were completely healed after 8 weeks.

## DISCUSSION

The protection provided by the bone structures of the sternum, mandible and cervical spine to the larynx skeleton and their own mobility cause laryngeal fractures to be seen rarely [9,10]. The most common causes of laryngeal fractures are motor vehicle accidents, sports-related trauma, assault and drowning [9,11]. In cases where there is a high velocity effect directly on the larynx, laryngeal fracture may occur due to the pressure of the larynx on the spine [12]. In addition, although it is rare, fractures can also be seen due to hyperflexion/hyperextension movements of the neck, apart from the direct effect of trauma [1].

Hoarseness is one of the most important symptoms in thyroid cartilage fractures, conservative treatment is preferred if there is no large hematoma around the fracture, and tracheostomy is applied in cases where there is a large hematoma that causes compression in the airways and extensive damage to the vocal cords [1,12]. In the present case, there was mild swelling and bleeding focus in the vocal cords. Tracheostomy was not performed because the lesions regressed in the follow-ups, conservative treatment was applied.

In forensic medicine practices, the mechanism of injury is important for elucidating the event [2]. In this case, injury occurred as an indirect effect of trauma to the neck region and it was determined that it was life-threatening due to the damage. The case we presented is compatible with the literature findings in terms of symptoms, diagnosis and treatment. It is presented because it is a rare case in forensic medicine practices in our country due to the mechanism of case formation and the type of injury.

### Conflict of interests

The authors declare that there is no conflict of interest in the study.

### Financial Disclosure

The authors declare that they have received no financial support for the study.

### Ethical approval

Ethics committee approval is not required.

### Acknowledgment

We respectfully commemorate Dr. Nusret AYAZ, who lost his life in the 6 February 2023 Malatya Earthquake and contributed to this article.

Presented as an oral presentation at the First International Medical Records Congress.

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## Review Article

# Evaluation of laser-induced breakdown spectroscopy (LIBS) applications in the aspect of forensic chemistry

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### Abstract

The most common definition of forensic science is the application of science to solve legal cases. In order to establish or rule out a connection between a suspect and a crime scene, forensic scientists analyze evidence collected from the crime scene, using multidisciplinary scientific methods (physics, chemistry, biology, mathematics, medicine, psychology, sociology, etc.). The identification of the sample and determination of whether two or more objects share a similar origin have been accomplished in forensic science using a variety of analytical techniques from past to present. However nowadays, with the developing technology, new methods that are more sensitive and easy to use are preferred. One of these techniques is Laser-Induced Breakdown Spectroscopy (LIBS). In forensic studies, LIBS is used to examine materials like counterfeit money, drugs, explosives, fingerprints, gunshot residue, ink, paper, hair, paint, glass, etc. On the other hand, it is more useful method compared to other methods due to the absence or simplicity of sample preparation, easy to operate, allowing the analysis of major, minor or trace elements and giving rapid results. This paper presents a review of the application of LIBS in forensic chemistry and the analytical results obtained with LIBS.

**Keywords:** Laser-induced breakdown spectroscopy, LIBS, forensic chemistry, elemental analysis

### INTRODUCTION

A wide range of premise chemical tests such as ninhydrin, luminol, etc. are used for the detection of visible or invisible bloodstains, drugs, semen or various traces (fingerprints, footprints, tyre marks, etc.) at the crime scene. In criminal laboratories, evidences from crime scenes are analysed microscopically by Scanning Electron Microscopy (SEM), elementally by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), and molecularly by Gas Chromatography-Mass Spectrometry (GC-MS) or Raman

Spectroscopy. However, these analytical instruments do not allow direct analysis of samples at the crime scene. Therefore, there is a need to develop new forensic tools that balance factors such as cost per analysis, ease of use, portability of the device, amount of sample consumed, sample preparation time, and efficiency. Laser-Induced Breakdown Spectroscopy (LIBS), which has recently emerged with the developing technology, is one of these forensic tools that have recently started to be used in forensic sciences.

The LIBS method utilizes a pulsed laser beam with high energy

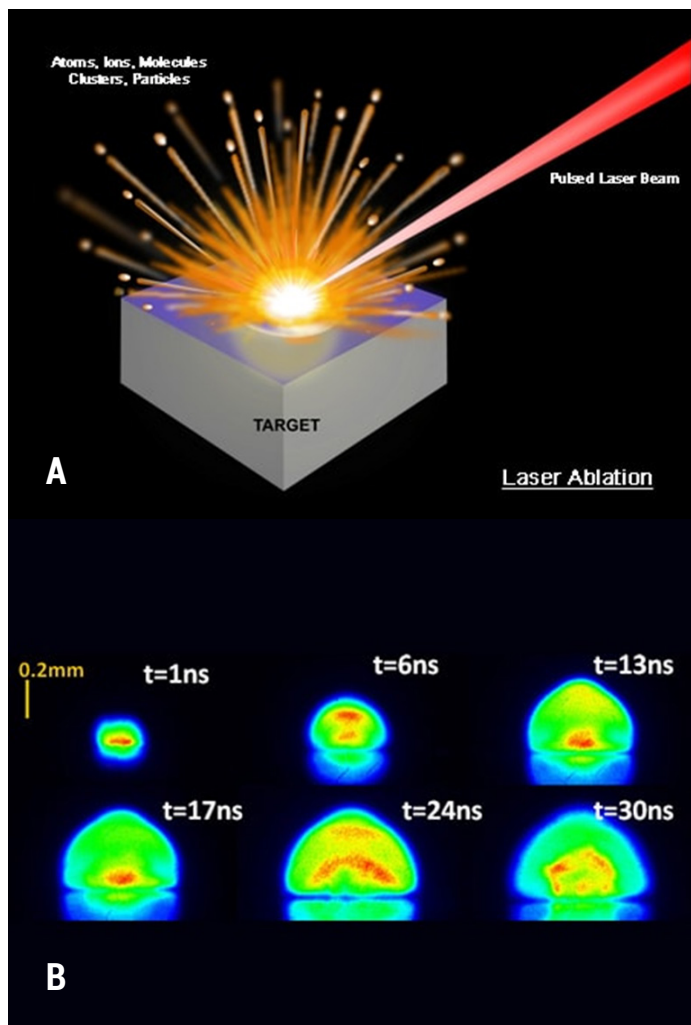
### CITATION

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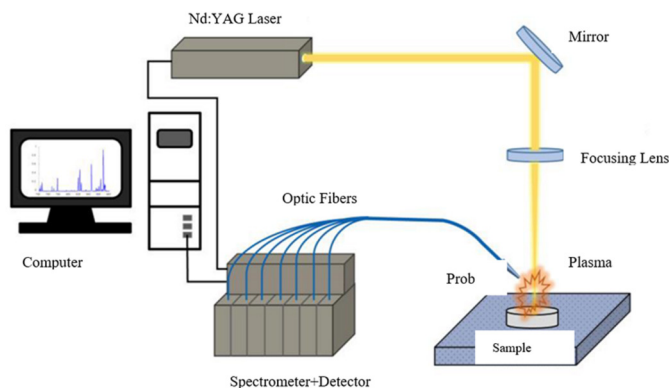
to generate a spark in the sample, thereby producing a plasma of high temperature that disintegrates molecules into atoms and stimulates the electrons in the atoms from a stable condition to an excited state. When the plasma cools, the excited electrons revert to their original state and emit light at atomic wavelengths that are characteristic [1]. On the other hand, LIBS is capable of being used to analyze sensitive elements like Al, Ba, Be, Ca, Cd, Cl, Cr, Cs, F, Fe, Mg, Mn, Na, Ni, Pb, Se, Ti, and V as well as elements with low atomic masses. Figure-1 illustrates how the laser interacts with sample and creates plasma.



**Figure 1A.** the pulsed laser strikes the sample's surface and creates a spark, **1B.** the creation of a high-temperature ( $>15,000\text{K}$ ) plasma above the sample surface and it expands quickly into the surrounding medium [2]

Although the laser was invented in the 1960s, its use in forensic sciences started in the early 2000s. Looking at the historical development of LIBS, the first functional laser was created using ruby crystal by Maiman in May 1960 [3]. After the discovery of the laser, the first laser-generated plasma spectroscopy ruby laser study was performed by Brech and Cross [4]. In 1963, plasma formation in gas media was first reported by Debras-Guédon and Liodec [5]. One year after that, Runge and colleagues employed a ruby laser that pulsed with a Q-switch to analyze metals [6]. Griem

published a book on plasma spectroscopy and followed in 1974 with an important thesis on the spectral line extended by plasmas [7]. By 1984, spectrochemical analysis of liquids was performed with LIBS [8]. Anglos et al. analysed pigments in works of art using LIBS [9]. In 2000, NASA started to work on LIBS for analysis on Mars, and in 2012, the first data from Mars were collected using LIBS [10].



**Figure 2.** Instrumentation of LIBS

In the experimental setup of LIBS consists of pulsed laser, focusing lenses, sample holder, spectrometer, detector, and computer for data analysis. The setup is shown in Figure-2. A high-energy and intense laser system is required for LIBS. Lasers are divided into pulsed lasers and continuous lasers. Pulsed lasers are classified according to their pulse times as nanoseconds, picoseconds, and femtoseconds. At the same time they are divided into three groups UV-laser, Vis-laser and IR-laser according to their wavelengths. Continuous lasers include He-Ne, CO<sub>2</sub>, and diode lasers [11]. However, the most widely used laser in LIBS is 1064 nm Nd: YAG laser in the near infrared region. The laser energy of the LIBS required for plasma generation should be between 10 $\mu\text{J}$ -500mJ, the pulse interval should be 100fs-10ns, and Nd:YAG (1064 nm) or its harmonics should be used. The LIBS spectrometer refracts plasma light and has various designs including Littrow, Paschen-Runge, Echelle, and Czerny-Turner, with the latter being the most commonly used. This spectrometer has an entrance slit, two mirrors, a diffraction grating, and a detector whose type depends on the intended application. Light passes through the slit and is collected by the first mirror, which then directs it to the grating. The grating reflects the light at different angles based on wavelength, and the second mirror focuses it onto the detector at the focal plane. The type of detector used can be an APD or PMT for intensity detection without spectral separation, or a spectrograph and PDA/IPDA for one-dimensional studies. For two-dimensional spatial data, CCD or ICCD devices are the most popular [12].

As with any analytical method, LIBS has its own set of limitations that need to be taken into account. For instance, the accuracy of the LIBS measurement may be reduced when the sample matrix is complex, since the emission intensity of the analyzed element is influenced by the presence of other elements. This implies that the same element at equal concentrations in two different samples may

display dissimilar LIBS emission intensities. Spectra acquired from a sample using different LIBS instruments may not necessarily match each other. Consequently, a spectral library generated using one LIBS instrument cannot be transferred to another. In comparison to other techniques like LA-ICP-MS, the sensitivity of LIBS is not as high, and it may not be possible to distinguish between similar samples with high precision. This limitation is due to factors such as self-absorption, spectral interferences, emission line overlap, and the fact that elements with high ionization potential (e.g. F, Cl, and S) have higher LODs [13].

LIBS has many advantages that make it preferable despite its disadvantages. The first of these is that sometimes no sample preparation is required and sometimes very few sample preparation steps are required. For example, solids can be analysed directly from the surface, no sample preparation step is required. Consumables are often required because it allows direct analysis of the sample. Since a small section/amount of the sample is studied for LIBS analysis, not the whole sample, it can be considered as non-destructive or minimally destructive. One benefit of LIBS is its ability to analyze all elements in the periodic table simultaneously using a single laser pulse, including elements with low atomic masses that are not accessible with certain other analytical methods. On the other hand, it is cheaper and faster than many other techniques. Finally, miniaturisation and ruggedisation of LIBS makes it portable, which is important for taking LIBS to the crime scene or near to evidence for direct analysis before the sample is contaminated [14].

With this new technology, elemental analysis of evidence such as soil, paint, fibre, firearm residue, explosives, documents, and ink has started to be performed in forensic laboratories. LIBS is also used for heavy metal analyses of biological samples such as hair, nails, blood and saliva.

### **Soil Analysis**

Soil contains organic and inorganic compounds in various proportions. Analysis of soil composition can provide information about the geological origin, dominant vegetation, and environment. Soil compositions can occur naturally or can be created in the laboratory environment. Therefore, soil evidence is important in forensic sciences in terms of linking the crime scene and the suspect. Soil particles can be transported from one region to another region by persons and/or individuals. With this transportability feature, it is important in terms of evidence in forensic sciences. Analysing the soil obtained as evidence in crime scene such as murder, theft, smuggling, terrorism, traffic accidents or sexual assault is important for the solution of the case. Soil, can be analyzed according to chemical composition and morphology of soil particles by Transmission Electron Microscopes TEM and Scanning electron microscopes SEM. Also both X-ray fluorescence and X-Ray Powder Diffraction XRD approach have both demonstrated great accuracy in determining the provenance of the soil. On the other hand, the elemental composition of soil can be analyzed by LIBS. There are several articles about using LIBS for soil examination. The first research on using LIBS for forensic soil analysis was

conducted by Martin et al. The article by Martin et al. (2007) discusses the potential of laser-induced breakdown spectroscopy (LIBS) as a high-resolution technique for environmental and forensic applications. The authors present the results of several experimental studies that demonstrate the applicability of LIBS for the analysis of various samples, including soil, plants, and forensic evidence. The experimental part of the article outlines the details of the LIBS setup used in the experiments, including the laser, spectrometer, and sample preparation. The authors describe how the laser was used to generate a plasma on the surface of the sample, and how the resulting emission spectra were recorded and analyzed using a spectrometer. They also discuss the challenges associated with LIBS analysis, such as spectral interferences and matrix effects, and how these challenges were overcome in the experiments. The authors present the results of their experiments in the form of several case studies. In one study, they used LIBS to analyze soil samples for heavy metal contamination. The results showed that LIBS was able to detect several heavy metals with high accuracy, demonstrating the potential of the technique for environmental monitoring applications. In another study, they used LIBS to analyze plant tissues for elemental composition. The results showed that LIBS was able to detect several elements, including potassium, calcium, and iron, at high spatial resolution, demonstrating the potential of the technique for plant physiology and ecology studies. The authors also present the results of several forensic case studies, demonstrating the potential of LIBS for forensic analysis. In one case, they used LIBS to analyze gunshot residue on clothing and skin samples, and were able to detect lead, barium, and antimony at high sensitivity. In another case, they used LIBS to analyze ink on a forged document, and were able to detect differences in the elemental composition between the forged and genuine documents. In conclusion, the authors demonstrate the potential of LIBS as a high-resolution technique for environmental and forensic applications. The authors also acknowledge the limitations of LIBS, such as the need for careful calibration and the potential for matrix effects, and suggest that further research is needed to fully realize the potential of the technique [15].

The article by S.C. and J.R.A. (2011) presents an investigation of the potential of laser-induced breakdown spectroscopy (LIBS) for the analysis and forensic characterization of soil samples. LIBS is a promising technique in forensic science due to its high sensitivity and non-destructive nature. The authors collected a set of soil samples from different sources, including natural soil, clay, and sand, to evaluate the performance of LIBS for soil analysis. The study was conducted using a Nd: YAG laser and a spectrometer, and the soil samples were ablated using a focused laser beam. The authors observed emission spectra for various elements, including silicon, aluminum, iron, calcium, magnesium, and potassium. They also developed a calibration curve for the analysis of silicon using LIBS. The results of the study showed that LIBS can be used to differentiate soil samples based on their elemental composition. The authors found that the emission spectra of soil samples collected from different sources were distinct and could be used to identify the source of the soil sample. The calibration curve

developed for silicon showed good linearity and low detection limits. The study also highlights the potential of LIBS for the analysis of other forensic samples such as hair and fibre, which could lead to further advancements in the field of forensic science. [16].

The article by Xu et al. (2020) presents a study on the use of laser-induced breakdown spectroscopy (LIBS) and Fourier transform infrared total attenuated reflectance spectroscopy (FTIR-ATR) for forensic soil analysis. The experimental part of the study involved collecting soil samples from different locations and analyzing them using both LIBS and FTIR-ATR. The authors then compared the results obtained from the two techniques to see how well they correlated. The study found that both LIBS and FTIR-ATR were effective in distinguishing between different soil types and that the two techniques complemented each other, with LIBS providing information on elemental composition and FTIR-ATR providing information on organic compounds. In their study, Xu et al. presented two case studies where they applied LIBS and FTIR-ATR techniques to forensic soil analysis. In the first case, soil samples were collected from a crime scene where the suspect was accused of burying a victim. The samples were analyzed using both LIBS and FTIR-ATR, and the results were compared to the control soil sample collected from a nearby location. The LIBS analysis identified differences in the elemental composition of the soil samples, which were used to distinguish the control soil from the crime scene soil. The FTIR-ATR analysis was used to compare the organic matter content of the soil samples, and the results also supported the differentiation between the two soil samples. In the second case, the soil samples were collected from the shoes of a suspect, who was accused of committing a hit-and-run crime. The soil samples from the shoes were compared to the soil samples collected from the crime scene using both LIBS and FTIR-ATR techniques. The LIBS analysis identified differences in the elemental composition of the soil samples, which were used to link the soil samples from the shoes to the soil samples from the crime scene. The FTIR-ATR analysis was used to compare the organic matter content of the soil samples, and the results also supported the link between the soil samples from the shoes and the crime scene [17].

### **Glass Analysis**

Glass samples can be important in forensic science for a number of reasons. First of all, glass fragments found at a crime scene can be linked to a suspect by comparing the physical and chemical properties of the glass with those found in the suspect's home or car. Furthermore, by examining the pattern of glass fractures and determining the direction of force that caused them, forensic scientists can reconstruct the sequence of events leading up to a crime. Another reason is that glass fragments can be used to identify the type of weapon used in a crime. For example, if a glass bottle was used to strike someone, the fragments left behind could indicate the type of bottle and potentially help identify the suspect. Lastly, glass fragments can also provide evidence of motive. For example, if a window was broken to gain entry into a building, the type of glass and the pattern of fractures

could provide clues about the perpetrator's motivation. In the end, glass analysis can be a valuable tool for forensic investigators in helping to link suspects to a crime, reconstruct the sequence of events, identify the type of weapon, and provide evidence of motive. The most popular analytical instruments used in glass analysis in forensic sciences include; Scanning Electron Microscope (SEM)- used for examining the surface morphology and elemental composition of glass fragments. Fourier Transform Infrared Spectroscopy (FTIR)- used for identifying the type of glass by examining the characteristic absorption bands of its components. X-ray Fluorescence Spectroscopy (XRF)- used for analyzing the elemental composition of glass fragments. Raman Spectroscopy- used for identifying the type of glass by examining the characteristic Raman spectra of its components. On the other hand, LIBS can also be used in forensic glass analysis.

The first paper in which LIBS was used in forensic glass analysis was published in 2006 by Bridge et al. The study aimed to evaluate the potential of laser-induced breakdown spectroscopy (LIBS) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) for the characterization of automobile float glass samples. In LIBS analysis, the samples were irradiated with a Nd:YAG laser and the spectra were collected in the 200-800 nm range. In LA-ICP-MS, the glass samples were ablated with a laser, and the resulting aerosol was analyzed using an ICP-MS instrument. The study showed that LIBS could be used to differentiate between the different types of glass used in automobile manufacturing, based on the presence of elements such as Si, Mg, Ca, and Al. The LA-ICP-MS analysis was able to provide quantitative information about the elemental composition of the glass samples, with detection limits in the low ppb range. The combination of these two techniques allowed for a more comprehensive characterization of the glass samples, providing information about both the elemental composition and the spectral properties of the samples. The study also investigated the potential of using LIBS and LA-ICP-MS for the forensic analysis of automobile glass samples. The results showed that these techniques could be used to provide information about the origin and history of the glass samples, by analyzing the elemental composition and isotopic ratios of the samples. The study demonstrated that the combination of LIBS and LA-ICP-MS could provide valuable information for forensic investigations involving automobile glass samples, such as hit-and-run cases or vehicle thefts. Overall, the study showed that LIBS and LA-ICP-MS are promising techniques for the characterization and forensic analysis of automobile glass samples. [18].

Bridge et al. handled a study on the forensic analysis of glass fragments using laser-induced breakdown spectroscopy (LIBS). The authors aimed to evaluate the potential of LIBS for comparative analysis of glass fragments in forensic investigations. The researchers collected a total of 134 glass fragments, which included both known and questioned samples. The known samples consisted of 16 glass types, while the questioned samples were collected from various sources such as hit-and-run incidents, burglary, and vandalism. The glass samples were analyzed using a LIBS system, which generated spectra that were used for

comparative analysis. The results of the study showed that LIBS analysis was capable of distinguishing between different types of glass, including tempered, laminated, and float glass. The LIBS system was able to detect trace elements present in the glass samples, which provided additional information for comparative analysis. The authors reported that the LIBS analysis was able to match questioned samples to known samples with a high degree of accuracy, making it a promising tool for forensic glass analysis. The authors also compared the results of LIBS analysis with those obtained from scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). The results showed that the LIBS system provided comparable results to the SEM and EDX techniques, indicating that LIBS analysis could be used as an alternative or complementary technique in forensic glass analysis. [19].

Yet another forensic glass study aimed to distinguish between different types of Australian window glass. The article by El-Deftar et al. discusses the forensic application of laser-induced breakdown spectroscopy (LIBS) for the discrimination of Australian window glass. The study aims to evaluate the potential of LIBS as a rapid and non-destructive technique for forensic glass analysis. The researchers collected 82 samples of window glass from various locations in Australia, including homes, businesses, and public buildings. The glass samples were analyzed using LIBS, and the spectral data were processed using principal component analysis (PCA) to identify potential groupings within the data. The results showed that LIBS was able to discriminate between different types of window glass, including float glass, tempered glass, and laminated glass, with a high degree of accuracy. In addition, the researchers also investigated the effects of different experimental parameters, such as laser energy and number of laser shots, on the LIBS analysis of glass samples. The results showed that increasing the laser energy and number of shots improved the sensitivity of the technique, but also increased the potential for sample damage. The findings of this study could have important implications for forensic glass analysis in Australia, particularly in cases where the identification of glass fragments is crucial for the investigation of crimes. The study demonstrates the potential of LIBS as a complementary technique to traditional glass analysis methods, and highlights the need for further research to fully explore the capabilities of the technique in a forensic context. [20].

The article describes an interlaboratory study that evaluates the interpretation of forensic glass evidence using refractive index (RI) measurements and elemental composition analysis. Glass samples from six different sources were analyzed using both techniques by seven different laboratories. The results showed a high degree of agreement between the laboratories for the elemental composition analysis, but a lower degree of agreement for the RI measurements. The study also revealed that the accuracy of the RI measurements was affected by the type of instrument used and the operator's experience level. The article discusses the importance of forensic glass analysis in criminal investigations and the challenges associated with interpreting glass evidence. The authors emphasize the need for objective and standardized

methods for the analysis and interpretation of glass evidence, and the interlaboratory study represents a step towards achieving this goal. The study also highlights the importance of quality assurance measures to ensure the reliability of forensic glass analysis results. The authors provide a detailed description of the analytical methods used in the study, including sample preparation, instrumentation, and data analysis. They also present the results of the study, including the degree of agreement between laboratories for both RI measurements and elemental composition analysis. The article concludes with a discussion of the limitations of the study, including the small sample size and the fact that the glass samples analyzed were not representative of all possible sources of forensic glass evidence. Overall, the article provides valuable insights into the challenges associated with interpreting forensic glass evidence and the importance of standardized analytical methods and quality assurance measures. The results of the interlaboratory study can inform the development of best practices for forensic glass analysis and improve the reliability and validity of glass evidence in criminal investigations. [21].

### **Paint Analysis**

Paint analysis is an important tool in forensic science as it can provide crucial information in the investigation of various crimes, including hit-and-run accidents, vandalism, burglary, and homicide. The analysis of paint samples collected from a crime scene can provide information about the type, origin, and source of the paint, as well as the make and model of the vehicle involved in a hit-and-run accident. The analysis can also help to link a suspect to the crime scene by identifying the presence of paint particles on their clothing or vehicle. Additionally, paint analysis can provide important information about the sequence of events that occurred during a crime, such as the order in which vehicles collided or the direction of impact. Overall, the analysis of paint samples can provide valuable evidence that can help to identify suspects, reconstruct crime scenes, and ultimately aid in the pursuit of justice. Additionally, paint analysis plays an important role in forensic science when it comes to identifying fake artwork. By analyzing the pigments, binders, and other chemical components in a paint sample, forensic experts can determine whether a painting is authentic or a forgery. For example, if a painting is claimed to be from a certain time period, the paint can be analyzed to see if the pigments used were actually available during that time. Additionally, if a painting is claimed to be by a certain artist, the paint can be compared to known samples of that artist's work to see if it matches. Paint analysis can also reveal evidence of overpainting or other alterations to a painting, which can be indicators of forgery. Overall, paint analysis is an important tool in the fight against art fraud and the preservation of cultural heritage.

This study investigates the applicability of Laser-Induced Breakdown Spectroscopy (LIBS) in the identification of pigments used in works of art. Pigments in pure pigment standards and painted artworks samples were analysed. A spectroscopic database containing analytical spectroscopic information was developed and used to solve the counterfeit artwork case. The

first step of the study is the systematic investigation of pigments using LIBS spectroscopy to identify their elemental composition. The resulting data has been compiled into a searchable database that includes pigment name, color, chemical structure, spectral analytical lines, and experimental parameters. Analysts can search the database using specific criteria, such as peak wavelengths or individual pigment spectra, to identify pigments or obtain information on specific elements and their spectral lines. The database provides guidance on selecting the appropriate spectral range and experimental conditions for measuring pigments. Several byzantine icons were examined during this study in order to identify pigments and verify to the originality of artworks. Egg tempera on wood late 16th-century icon that depicted the Virgin Mary holding Jesus was analyzed, with a focus on the white, green, and red painted areas. Lead was identified (357.27, 363.96, 368.35, 373.99, and 405.78 nm) in the white pigment, which was expected due to the age of the icon, suggesting that lead white was the favored pigment during that time. Copper emission lines (510.55, 515.32, and 521.82) were detected in the green paint, indicating that the pigment used was likely malachite. The red paint's LIBS spectrum showed emission lines from iron, aluminum, and calcium, indicating that the pigment used was an iron-based red, such as sienna or hematite. Also, an icon of Saint Nicholas from the mid-19th century was studied, which was created with egg tempera on silver foil. As anticipated, the original white pigment used was lead white. However, when examined a different area of the icon, observed the characteristic emission from zinc (328.23, 330.26, 334.50, 468.01, 472.21 and 481.05) in the first LIBS spectrum, which suggested that zinc white was used. Further analysis with three more laser pulses on the same point showed the presence of lead, indicating that the zinc white layer was applied as an overpaint on top of the original lead white paint layer. After forty laser pulses on the same point, only calcium (393.37, 396.85, 422.67, 443.50, 445.48, 527.03, and 558.20) was found, indicating that the preparation layer, most likely composed of calcite or gypsum, had been reached. This case study is an example of the usefulness of LIBS in determining the successive paint layers' depth profile. The laser pulses remove a small amount of material from the surface during each analysis, which allows each subsequent pulse to probe a slightly deeper layer, revealing the paint layers' stratigraphy and conducting an in-situ cross-section analysis. The researchers also studied an icon from the 16th century, depicting The Annunciation. Analysis of the red paint revealed the presence of mercury (365.02, 404.66, 435.83, 546.07, 576.96, and 579.07) through the identification of characteristic peaks, indicating that cinnabar (HgS) was used as the pigment. When analyzing a yellow pigment, the LIBS spectrum showed atomic emission lines for lead, chromium (357.87, 359.35, 360.53, 425.43, 427.48, 428.97, 520.45) and calcium. This suggests that lead chromate, also known as chrome yellow, was used on the icon. However, the use of chrome yellow raises questions about the authenticity of the paint since it was not introduced until the mid-18th century, whereas the icon dates back to the 16th century [22].

McIntee et al. (2010) investigated the use of laser-induced breakdown spectroscopy (LIBS) for the comparative analysis of automotive paints. The authors analyzed the elemental composition of paint samples from four different car manufacturers using LIBS and compared the results using nonparametric permutation tests. The study involved the analysis of 21 different automobile paint samples, each of which was divided into two groups. The first group was used for calibration purposes, while the second group was used to test the accuracy and precision of the techniques. The LIBS measurements were taken using a Nd: YAG laser operating at 1064 nm, while the nonparametric permutation tests were conducted on the intensity data obtained from the LIBS measurements. The results showed that both techniques were capable of distinguishing between the different paint samples. However, the LIBS technique was found to be more accurate and precise, with lower measurement uncertainties and better performance in terms of sensitivity and specificity. The authors also noted that the LIBS technique was faster and more cost-effective than the nonparametric permutation tests. The authors attributed the improved performance of the LIBS technique to its ability to detect a larger number of elements and isotopes, as well as its higher spatial resolution. The LIBS technique was able to detect a range of elements including carbon, oxygen, magnesium, aluminum, silicon, calcium, titanium, and iron. The analyst also evaluated the potential of using the LIBS technique to analyze paint samples from different areas of a vehicle, such as the hood, door, and bumper. They found that the technique was able to distinguish between the different areas with high accuracy and precision. In the end, McIntee et al. (2010) demonstrated that the LIBS technique is a powerful tool for the forensic analysis of automotive paints. The technique was found to be highly accurate, precise, sensitive, and specific, making it suitable for use in both qualitative and quantitative analyses of paint samples. [23].

### **Ink Analysis**

Ink analysis is an important field within forensic science because it allows investigators to determine the origin and properties of ink used in a document. This information can be used to determine the authenticity of a document, to link different documents to a particular source or person, and to identify alterations or forgeries. Ink analysis can reveal a variety of characteristics about the ink used in a document, including its chemical composition, the age of the ink, and the type of pen or printer used to create the document. This information can be compared to known standards and databases to identify potential matches or inconsistencies. Forensic scientists can also use ink analysis to identify similarities or differences between different documents, to determine if they were created by the same person or if they were created using different methods or materials. This information can be critical in criminal investigations, where the authenticity of documents can play a crucial role in solving a case. In general, ink analysis is an important tool in forensic science because it allows investigators to gather valuable information about the origin and properties of documents, which can help them to make informed decisions in

criminal investigations and other legal proceedings.

The study by Kula et al. (2014) investigates the potential of Laser-Induced Breakdown Spectroscopy (LIBS) for forensic analysis of writing inks. The authors aim to determine whether the technique can be used to differentiate between inks from different sources and to provide information about the elemental composition of the inks. To conduct the research, the authors analyzed a range of ink samples using LIBS, including ballpoint, gel, and liquid inks from different manufacturers. The results showed that the technique was able to differentiate between inks from different sources with a high level of accuracy. Additionally, the technique provided elemental composition data for the inks, including the identification of trace elements that are characteristic of specific inks. The study also examined the effect of different writing surfaces on the accuracy of the LIBS analysis. The authors found that the surface material did not significantly impact the accuracy of the technique, and that the technique could be applied to ink samples on paper without any prior sample preparation. One limitation of the study is the relatively small sample size used for analysis. However, the authors suggest that the results provide a promising foundation for further research into the use of LIBS for forensic analysis of writing inks. Future research may explore the application of LIBS to other types of ink samples, such as those found in printing or photocopying. Overall, the study demonstrates the potential of LIBS for forensic analysis of writing inks. The technique provides accurate information about the elemental composition of inks, and can be used to differentiate between inks from different sources. These findings suggest that LIBS may have significant potential for use in forensic investigations, particularly in cases where ink samples are limited or where traditional ink analysis methods are not feasible. In conclusion, Kula et al. (2014) provide valuable insight into the potential of LIBS for forensic analysis of writing inks. The study suggests that the technique may offer a promising alternative to traditional ink analysis methods, and could have significant implications for forensic investigations. Further research is needed to fully explore the potential of the technique, but the results of this study provide a solid foundation for future investigations. [24].

In their 2020 study, Cicconi et al. examine the feasibility of LIBS for the discrimination of inks used in documents, with a focus on the ability to differentiate between inks from different manufacturers. The study uses a laser to create a plasma from the ink samples, which emits characteristic spectra that can be analyzed to identify the elements present in the ink. The authors conduct their research on five different commercial inks, representing different types and manufacturers. They analyze the inks using LIBS and compare the resulting spectra to identify differences and similarities between them. The authors found that LIBS is a powerful technique for the forensic analysis of inks. They were able to differentiate between inks from different manufacturers based on their spectral fingerprints. The authors also explored the effect of various factors, such as the number of laser shots and the laser energy, on the accuracy of the analysis. They found that increasing the

number of shots and energy could improve the accuracy of the analysis. The technique could be used to identify the origin of a document or determine whether a document has been altered. The authors also suggest that the technique could be used to identify counterfeit products by analyzing the inks used in their packaging. In conclusion, the study demonstrates the potential of LIBS as a powerful tool for the forensic analysis of inks. The authors show that the technique is capable of differentiating between inks from different manufacturers based on their spectral fingerprints. The technique has implications for the identification of counterfeit products, as well as the detection of document alterations. Overall, the study highlights the importance of advanced analytical techniques in forensic science and the potential of LIBS for future applications in this field. [25].

The article discusses the application of LIBS and PCA for the analysis of printing ink. The study focuses on the analysis of commercial inks using LIBS and PCA. The authors use a laser to create a plasma from the ink samples, which emits characteristic spectra that can be analyzed to identify the elements present in the ink. They then use PCA to analyze the resulting spectra and identify differences and similarities between the ink samples. It was found that the combination of LIBS and PCA is a powerful tool for the analysis of printing ink. They were able to differentiate between ink formulations based on their spectral fingerprints and identify counterfeit products. They also showed that the technique could be used to determine the age of ink by analyzing the changes in the ink composition over time. The study has important implications for the printing industry and forensic science. The technique could be used to prevent the circulation of fake products in the market, monitor the quality of ink formulations, and ensure consistency in ink production [26].

### **Biological Sample Analysis**

Analysis of biological samples is crucial in forensic science. The first reason of it is that biological samples, such as blood, saliva, and hair, can be used to identify individuals involved in a crime scene. DNA analysis is commonly used to match biological samples found at the scene to suspects or victims, providing strong evidence for or against a particular individual's involvement. Other than that, biological samples, particularly tissue and blood samples, can be used to determine the time of death of a victim. This information can be important in reconstructing the events that led to the victim's death and identifying potential suspects. Also, it can be used to identify the cause of death, particularly in cases where toxicological analysis is required. This information can be important in determining if the death was due to natural causes, an accident, or a homicide. Finally, biological samples can provide information on the age and gender of an individual, particularly in cases where the body is severely decomposed or skeletal remains are found.

On the other hand, elemental analysis of biological samples also is an important tool in toxicology and forensic science. Begin with, elemental analysis can help identify the presence of toxic elements,

such as lead, mercury, and arsenic, in biological samples. These elements can be toxic even at low concentrations and can cause a range of health effects, so their identification is critical in assessing exposure and potential toxicity. Second of all if an individual has been exposed to toxic elements in the environment, such as in contaminated water or air, elemental analysis can help determine. This information can be important for evaluating health risks and for assessing the effectiveness of public health interventions. Another reason elemental analysis can help monitor workers who are exposed to toxic elements on the job, such as lead in battery manufacturing or mercury in dental fillings. Regular monitoring can help identify exposure levels and prevent long-term health effects. Elemental information can be helpful to identify the remains of an individual in cases where traditional identification methods are not possible. For example, by analyzing the elemental composition of bones or teeth, forensic scientists can determine the age, gender, and geographic origin of the individual.

LIBS can identify and quantify trace elements in biological samples such as blood, hair, and tissue. These elements can provide valuable information about the individual, including their geographical origin, lifestyle, and exposure to toxins or drugs. Unlike other analytical techniques, such as gas chromatography or mass spectrometry, LIBS does not require sample preparation or destruction. This means that the sample can be preserved for further analysis or used as evidence in court. It can provide rapid analysis of biological samples, which is critical in forensic investigations where time is of the essence. The analysis can be performed in real-time, and the results can be obtained within seconds. That's why LIBS is an important analytical technique for the elemental analysis of biological samples.

The article examines the differences in the elemental composition of fingernails between healthy individuals and those who are addicted to opium. The study uses LIBS to analyze fingernail samples and compare the results between the two groups. The research aims to provide insights into the impact of opium addiction on the human body, specifically its effect on mineral absorption and metabolism. By analyzing the elemental composition of fingernails, the authors can investigate the levels of minerals present in the body, as nails reflect the composition of the bloodstream. The study found that there are significant differences in the elemental composition of fingernails between healthy individuals and those addicted to opium. Specifically, the levels of calcium, magnesium, and iron were lower in the fingernails of opium-addicted individuals than in those of healthy individuals. The findings suggest that opium addiction can negatively impact the body's ability to absorb and metabolize minerals. The analysis of fingernail samples using LIBS is a useful tool for addiction research and could potentially be used to monitor the progress of addiction treatment programs. The results demonstrate significant differences in mineral levels between healthy individuals and those addicted to opium, underscoring the impact of addiction on the body's ability to absorb and metabolize minerals [27].

The study by Moncayo et al. (2014) provides a promising

approach to identifying human remains, particularly in forensic investigations where traditional methods may not be reliable or take an extended period. The authors collected samples from eight individuals, which included bones, teeth, and soft tissue remains. They used a Nd:YAG laser to generate plasma from the samples and record their spectral emission using an optical spectrometer. The spectral data were then analyzed using a neural network algorithm to differentiate between human bodies, bones, and teeth remains. The authors noted that bone and teeth remains' spectral data were similar, making it difficult to differentiate between the two using traditional methods. However, the neural network was effective in identifying subtle differences in the spectral data, allowing for accurate classification of the samples. The speed of the analysis is also essential in forensic investigations where time is of the essence. Traditional methods of identification can be time-consuming, requiring DNA analysis or dental records, while the approach by Moncayo et al. (2014) takes minutes. The results showed that the neural network was able to accurately discriminate between soft tissue, bone, and teeth samples, with an overall accuracy of 96.7%. The researchers also found that certain spectral lines were particularly useful for discrimination, such as calcium and magnesium lines for bones, and carbon and nitrogen lines for soft tissue. The study's findings have significant implications for forensic investigations, particularly in scenarios where traditional methods of identification are not possible or reliable. The technique can be used to identify victims of natural disasters, mass graves, or accidents where identification is challenging due to fragmented remains. The approach by Moncayo et al. (2014) also has applications in archaeological studies, where the preservation of remains is essential for further analysis [28].

### **Gunshot Residue Analysis**

Gunshot residue (GSR) and ammunition analysis are essential components of forensic science as they can provide valuable information in criminal investigations involving firearms. GSR analysis involves the identification and characterization of microscopic particles discharged from a firearm upon firing, such as primer residues, gunshot primer (GSP) particles, and propellant residues. These particles can be found on the shooter's hands, clothing, and other surfaces in the vicinity of the shooting. GSR analysis can provide important evidence to link a suspect to a crime scene, determine the type of firearm used, and reconstruct the sequence of events leading up to the shooting. Ammunition analysis, on the other hand, involves the examination of ammunition components, such as bullet casings, projectiles, and cartridge cases, to determine their origin, manufacturing method, and potential association with a particular firearm. This analysis can help to establish the type of firearm used, identify suspects, and provide information about the number of shots fired. In summary, GSR and ammunition analysis are critical tools for forensic scientists in firearm-related investigations, providing valuable information for criminal investigations and helping to bring justice to victims and their families.

Silva et al. (2009) handled a study on the screening analysis of



gunshot residues using LIBS. It was aimed to investigate the potential of LIBS for detecting and characterizing gunshot residues in real-time. The study involved the use of a Q-switched Nd:YAG laser to ablate samples of gunshot residues collected from different surfaces. The LIBS spectra of the ablated residues were then recorded and analyzed using multivariate statistical techniques. According to analyzed the spectra obtained from the GSR samples and identified several elements that are commonly found in GSR, including lead, barium, antimony, and copper. They also found that the intensity of the spectral lines for these elements varied depending on the distance between the laser and the GSR sample. Also, it was found that the spectral patterns of gunshot residues were distinct from those of other common environmental and industrial contaminants, such as soil and metal. On the other hand, the effect of different experimental parameters on the LIBS analysis of gunshot residues, such as the laser energy and the number of laser pulses was also investigated. They found that higher laser energies and more laser pulses led to better spectral quality and improved detection limits. However, the authors also acknowledged that there are some limitations to the use of LIBS for GSR analysis. One limitation is that the technique is not as sensitive as traditional methods, meaning that it may not detect low levels of GSR. Additionally, the authors noted that LIBS may not be able to distinguish between GSR and other types of particles that may be present at a crime scene. It was suggested that further research is needed to address these limitations and to determine the accuracy and reliability of LIBS for GSR analysis [29].

The study conducted by Doña-Fernández et al. (2018) focused on comparing two different methods of detecting gunshot residue (GSR) particles at a crime scene. These methods include scanning electron microscopy with energy dispersive X-ray analysis (SEM/EDX) and a portable laser-induced breakdown spectroscopy (LIBS) system. The aim of the study was to determine the efficiency and accuracy of these methods in detecting GSR particles in real-time. The study compared SEM/EDX and a portable Laser-Induced Breakdown Spectroscopy (LIBS) system in terms of sensitivity, specificity, and reliability. The authors collected 30 samples from different firearms discharge scenarios and analyzed them with both techniques. The SEM/EDX results showed that all 30 samples contained lead, barium, and antimony particles, while the portable LIBS system detected these particles in only 22 samples. However, the portable LIBS system showed a higher sensitivity to barium and antimony than SEM/EDX. The study also evaluated the reliability of the two techniques in identifying GSR particles on clothing fabrics. The SEM/EDX technique provided more detailed information about the particles' morphology, while the LIBS system showed a better ability to analyze samples in situ. Overall, the study found that both SEM/EDX and portable LIBS systems have advantages and limitations, and the choice between them depends on the circumstances of each case. The SEM/EDX technique provides more detailed information about the particles' morphology, which can be useful in identifying the source of the gunshot, but it requires a specialized laboratory and a longer analysis time. On the other hand, the portable LIBS system can

provide real-time results and can be used in situ, but it has a lower sensitivity to lead particles. The study concludes that the portable LIBS system has great potential as a complementary technique to SEM/EDX in the analysis of GSR particles. The combination of both techniques can provide more complete and reliable information about the particles' composition and morphology, improving the accuracy of forensic investigations. [30].

## Other Studies

### Nuclear Debris Analysis

Nuclear forensics is the field of forensic science that deals with the analysis of nuclear and other radioactive materials to provide evidence and attribution for nuclear events such as nuclear weapons testing, nuclear accidents, illicit trafficking of nuclear materials, and nuclear terrorism. It involves the application of a wide range of scientific techniques to identify and characterize nuclear materials, such as isotopic analysis, spectroscopy, and microscopy, among others. The goal of nuclear forensics is to determine the origin, history, and intended use of nuclear materials and to support law enforcement and national security efforts to prevent and respond to nuclear-related incidents. Rapid action is essential as a result of a nuclear or radioactive incident in order to contain the radioactive material, reduce radiation risks at the place of the incident, and keep nuclear forensic evidence that can be used to identify the source and method of production. Gamma-ray spectroscopy and neutron detection are the current techniques used for on-site analysis of radioactive material in nuclear forensics, but they have obstacles for determining alpha- and beta-emitting isotopes as well as non-radioactive impurities. LIBS, which is quick and flexible enough to work in a variety of situations, can be used in nuclear investigations to get around these restrictions.

Lee (2018) proposes a new approach to post-detonation nuclear forensics that involves on-site laser-induced breakdown spectroscopy (LIBS) analysis in real-time. Lee's proposal involves using LIBS to analyze samples collected from the site of a nuclear detonation. This would allow for real-time analysis of the debris, providing crucial information about the origin and characteristics of the nuclear material used. In particular, LIBS could be used to identify the isotopic composition of the material, which can provide important clues about its source. This could help to deter future nuclear threats and hold responsible parties accountable for their actions. To validate the potential of the proposed approach, Lee conducted a series of experiments to evaluate the accuracy and precision of LIBS analysis for nuclear forensics. The experiments involved analyzing standard samples of uranium and plutonium, as well as simulated nuclear debris samples created using a laser ablation technique. The results demonstrated that LIBS analysis was able to accurately identify the isotopic composition of the samples, with a precision comparable to other analytical techniques. Overall, Lee's proposal for on-site LIBS analysis for nuclear forensics represents an innovative approach to a pressing problem. While there are some challenges to implementing this technique in practice, the potential benefits of real-time analysis of nuclear debris samples are significant. As the threat of nuclear

terrorism continues to grow, new approaches to nuclear forensics will be essential for ensuring global security. On-site LIBS analysis is a promising avenue for further research and development in this important field [31].

### **Fingerprint Analysis**

Fingerprint analysis has long been a cornerstone of forensic investigation, helping to identify suspects and link them to crime scenes. However, traditional fingerprint analysis techniques can be limited in their ability to discriminate between prints and provide conclusive evidence. Yang and Yoh (2018) propose a new approach to fingerprint analysis that involves using laser-induced breakdown spectroscopy (LIBS) and chemometric approaches to improve the discrimination and accuracy of fingerprint identification. The researchers employed a chemometric approach to analyze the LIBS data. Chemometrics is a field that involves using statistical and mathematical methods to analyze chemical data, and it has been used extensively in other areas of spectroscopy analysis. The authors used principal component analysis (PCA) and discriminant analysis (DA) to analyze the LIBS data and identify unique spectral features that could be used to discriminate between different fingerprints. The authors handled series of experiments to evaluate the accuracy and precision of their proposed approach. The experiments involved analyzing a set of latent fingerprints on various substrates, including glass, plastic, and metal. The results demonstrated that the proposed approach was able to accurately discriminate between different fingerprints and identify unique characteristics that could be used for forensic analysis. The success of the proposed approach was due to the unique chemical composition of the fingerprint residue. Fingerprint residue consists of a complex mixture of organic and inorganic compounds, including amino acids, fatty acids, and salts. These compounds have unique elemental compositions, and the LIBS technique can be used to detect these elemental compositions, which can be used to identify unique characteristics of individual fingerprints. One potential limitation of the proposed approach is the cost and accessibility of the equipment required for LIBS analysis. While the cost of LIBS instruments has decreased in recent years, they may still be prohibitively expensive for some forensic laboratories. Additionally, the complexity of the data analysis may require specialized expertise and software tools, which may not be readily available in all forensic laboratories [32].

### **Fire Debris Analysis**

In forensic science, fire debris analysis is an essential process that aims to identify and analyze the chemicals and residues left behind after a fire. This analysis helps forensic investigators to determine the cause and origin of the fire, which is crucial in both civil and criminal cases. The field of forensic science continues to advance as new technologies are developed and implemented in various areas of investigation. One area of forensic science that has seen recent advancements is the use of laser-induced breakdown spectroscopy (LIBS) in fire debris analysis. The study conducted by Choi and Yoh (2017) aims to demonstrate the feasibility of using LIBS

for fire debris analysis in forensic fire investigation. The authors collected fire debris samples from a controlled burn of a structure, including samples from the floor, wall, and ceiling. These samples were then analyzed using LIBS, and the resulting spectra were compared to a reference database of known substances. They also compared the results obtained from LIBS with those obtained using GC-MS and FT-IR. The results of the study showed that LIBS was able to accurately identify the substances present in the fire debris samples. The authors identified a number of elements and compounds present in the samples, including carbon, hydrogen, oxygen, nitrogen, and sulfur. These elements and compounds were consistent with those found in common construction materials, as well as potential accelerants such as gasoline and kerosene. It was found that the results obtained from LIBS were comparable to those obtained from GC-MS and FT-IR. The study concludes by emphasizing the potential benefits of using LIBS for fire debris analysis in forensic investigations. [33].

Also recent studies show that LIBS can be powerful tool for different field of forensic sciences such as writing marks [34], determining age and sex from tooth specimens [35], cultural heritage and archaeology [36] and investigation of textiles [37].

### **CONCLUSION**

Laser-Induced Breakdown Spectroscopy (LIBS) has proven to be a valuable analytical technique in forensic science. It has been widely used for the analysis of various types of evidence, such as soil, paint, ink, and gunshot residue (GSR), due to its non-destructive, rapid, and sensitive nature. LIBS has been used for the analysis of soil in forensic science to determine its elemental composition, which can help to identify the origin of soil samples. For instance, researchers have used LIBS to analyze soil samples from different locations in order to determine the elemental composition of each sample. By comparing the elemental composition of the soil samples, they were able to determine the source of soil samples found at a crime scene, which can be useful in linking suspects to a crime. Paint is another important type of evidence that is often found in crime scenes. It can provide valuable information about the type of vehicle or object involved in a crime. Researchers have used LIBS to analyze the elemental composition of paint samples collected from different sources, including different vehicles and objects. By comparing the elemental composition of the paint samples, they were able to determine the source of paint samples found at a crime scene, which can be useful in identifying the vehicle or object involved in a crime. Ink is also an important type of evidence that can be found in crime scenes, and its analysis can provide valuable information for forensic investigations. LIBS has used to analyze the elemental composition of ink samples collected from different sources, including different types of pens and printers. According to results, investigators were able to determine the type of ink used in a document, which can be useful in identifying the author of a document by comparing the elemental composition of the ink samples.

In conclusion, LIBS has proven to be a valuable analytical

technique in forensic science for the analysis of various types of evidence, including biological samples, fire debris or fingerprint. Its non-destructive, rapid, and sensitive nature makes it a useful tool for forensic investigations. The analysis of these types of evidence can provide valuable information that can be used to link suspects to a crime, identify the source of evidence, and determine the type of firearm used in a crime. While LIBS data analysis cannot fully replace traditional analytical methods such as gas chromatography, its application in forensic science can provide complementary information that can be useful in solving criminal cases.

#### Conflict of interests

*The authors declare that there is no conflict of interest in the study.*

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