Bones, Stories, and Broken Hearts: The Psychosocial Impact of Mass Grave Investigations on Surviving Families in Sorya, Duhok Governorate, Iraqi Kurdistan

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Abstract

Background and objectives:

A long history of war has resulted in thousands of missing people and families with ambiguous loss in Iraqi Kurdistan. The recent forensic exhumation in Sorya, Duhok, brought renewal to hope and opened old wounds. Although the Sorya graves have been forensically documented, little research has been conducted on psychosocial impact and family coping mechanisms. This paper examines how grief, resilience, and recognition and justice expectations are formed by bones, narratives, and legal processes involving affected families to inform culturally responsive psychosocial support and victim-focused forensic practice.

Methods: Hermeneutic phenomenology (van Manen) was used to study ten relatives (5 women, 5 men; 33 - 64 years) of mass-grave victims found in Sorya. Semi-structured interviews (65 - 85 minutes) were conducted from July to August 2025 to collect required data. Meaning units and themes were obtained by employing thematic analysis via van Manen's six activities.

Results: Five interlinked themes emerged: (1) the weight of uncertainty; (2) stories as carriers of grief and memory; (3) emotional landscape of exhumation; (4) coping and support systems; and (5) healing through recognition and justice.

Conclusion: Exhumation enables recognition but does not automatically heal the grief. Family-centered, transparent, culturally grounded forensic processes – paired with accessible, community-based psychosocial support and meaningful acknowledgment and accountability – are crucial to restore dignity and mitigate enduring harms.

Keywords: ambiguous loss; mass grave investigations; exhumation; Sorya village, Duhok Governorate, hermeneutic phenomenology; psychosocial support; transitional justice

Introduction

In Iraq and the Kurdistan Region, rumors of violence in the state and among insurgents have rendered thousands missing and numerous mass graves, in a dilemma of long-term predicament of the families. Recent forensic digs in Sorya, Duhok Governorate have rekindled hopes of identification as well as reviving old unhealed wounds (Amin & Othman, 2020). Ambiguous loss – the absence of certainty about death or survival – produces a distinctive, enduring distress characterized by oscillation between hope and fear and difficulties in mourning (Boss & Yeats, 2014). Increased risks of complicated grief, depression, and impairment are linked to such liminality, and can possibly persist even in cases of recovered remains, when

no meaning and recognition are achieved (Shear, 2015; Miller & Rasmussen, 2010).

To the families of the disappeared, stories and testimonies serve as essential transporters of memory keeping the social identities of the victims and the moral worlds of the survivors alive (Robins, 2011). Sharing narratives has the potential to act as a survival mechanism and at the same time influence the interpretation of the younger generations about violence and belonging, which has been extensively studied through intergenerational trauma in conflict-affected and displaced families (Hirschberger, 2018; Sangalang & Vang, 2017). However, the legal and forensic procedures concerning digging up and identification are emotional: the prospect of it, encountering tangible proofs of cruelty, and involvement with symbolic agents have the power to traumatize even when they are expected to be honest (Brounéus, 2010).

Coping occurs in family, faith, and community systems that tend to offset limited or stigmatized formal mental health provision, a frequent limitation in low- and middle-income contexts and in Iraq (Semrau et al., 2015). Meanwhile, the efforts needed to establish meaning, which include rituals of recognition, which consist of public acknowledgment, noble reburying, and rituals, can alleviate the need to create meaning, and demands of accountability would be part of healing (Robins, 2011; Shear, 2015). Despite the recovery and examination of human remains being described in the forensic studies by Sorya (Amin and Othman, 2020), little is known about the psychosocial experiences of the impacted families in the context of mass grave investigations.

This research is needed to have a record of how, in their words, bones, stories, and legal processes overlap to create grief and resilience among families of the victims found in Sorya. With a focus on the views of families, we seek to educate culturally sensitive psychosocial assistance and victim-focused forensic practice in the course of and following exhumations.

Methods

Study Design

The present qualitative study used the hermeneutic phenomenological technique developed by van Manen (1990). Such a methodological decision allowed knowing the experiences of the families of the victims of the mass graves effectively in Sorya, Duhok Governorate, Iraqi Kurdistan, and listening to their personal stories of memory, grief, and mental health.

Participants

In the present study, purposive sampling identified ten family members of mass grave victims at the Sorya site, located in Duhok Governorate in Iraqi Kurdistan (5 men and 5 women). Inclusion criteria included relatives of mass grave victims in Sorya, willingness to take part in the research, and firsthand experience. Those family members who did not decide to join the project were excluded. Ages ranged from 33 to 64.

Data Collection

The data were collected through semi-structured interviews that were conducted in July and August 2025. Interviews were conducted in the homes or other places where the participants were sure of their safety and comfort. Interviews continued until thematic saturation, that is not new themes emerged. Some of the interview questions are as follows:

- Could you tell me a little about your family member whose remains were identified?

- What memories or stories about them are most meaningful to you?

- What was it like for you and your family when they first disappeared or were killed?

- How did you first learn that a forensic exhumation was taking place?

- Did you feel that your cultural or religious traditions were respected during the exhumation and identification?

- How has this experience affected your emotional wellbeing?

- How did your community respond when your loved one was identified?

- What does this identification mean to you personally?

- What do you feel is still missing for your healing or justice?

- Is there anything I haven't asked that you think is important to share?

All interviews were recorded (subject to the permission of the participants) and were restricted to an average of sixty-five to eighty-five minutes. Thereafter, the tapes were translated into English and transcribed verbatim to analyze.

Data Analysis

Analysis of the data followed six methodological actions by Van Manen (1990). The primary researcher was a forensic, genocide, and Anfal researcher and member of the corresponding community, which implies that he based the investigation on the familiarity in intimate perspective during the first exercise, which involved the reorientation of the lived experience. The second step was to look at the experience in its experienced form as perceived by the family members and relatives. The third exercise, which was pegged into phenomenological thematic analysis, was found to have important themes. The fourth exercise involved the subjects talking on paper about what they had observed and then rewriting the handwritten spirits to have their voices recorded. As all subjects were backed by the experience of the participants, the issue of internal consistency was rated highly in the context of the fifth activity that was inclined towards the correct description of the phenomenon. Lastly, the parts and whole derived were stabilized by using the switch between the coded transcripts and the holistic themes (Van Manen, 1990).

Trustworthiness

Credibility to the results was provided using long-term commitment to the respondents, the member-checking method and debriefing of the findings by professional peers. The close data collection was facilitated and achieved by the friendly relationship between the participants and the researchers. The work also earned more credibility due to the extensive experience of the researcher in the field of forensics, genocide, and Anfal works. The texts presented by the participants were directly translated into interpretations out of the transcripts which were weighed several times.

Ethical Considerations

The study was approved by Hawler Medical University College of Nursing Ethics Committee (Project No. XX; Approval Date: DD MM YYYY). The participants were notified about the study purpose and its confidentiality rules and their rights, including the right to refuse to take part in the study. Each of the respondents signed a voluntary statement form and an informed consent form. For anonymity purposes, each respondent was assigned a code (P1, P2, etc.) that would keep all related digital or physical data secure.

Results

The grief of families of victims found in mass graves in Sorya is both intimate and public, and spread over time and record keeping. Their words help reveal the role that uncertainty, memory, and the pursuit of justice play in the everyday life of mass killing survivors. These voices express the textures of waiting; the strength of narration and the little hope exhumation and recognition can present. Collectively, they chart the emotional landscape of grief, courage and dignity searching.

Main Theme 1. The Weight of Uncertainty

The definition of uncertainty was a daily companion that dripped into routines, decisions, and relationships. Life was held in suspension with no reassurance that the world had also stalled but still moved.

"It's this question every morning I wake up asking: where is he? The not-knowing sits on my chest heavier than stone." – P6

"We trained to live in-between phone calls and rumors not breathing full. Even joyous situations become borrowed in case the answer is not provided

yet." – P3

Subtheme 1.1. Living in limbo

People used to refer to an in-between state which was exhaustive and confusing before identification. Mourning felt forbidden, and hope felt fragile.

"We keep his clothes folded in the cupboard because closing it feels like closing the door on him. My mother asks me to leave the light on, as if it might guide him home." – P8

"I can't say 'was' or 'is' when I speak of my mother. It's like my tongue refuses to choose a side." – P1

Subtheme 1.2. Hope versus fear

Families described a tug-of-war between wanting truth and fearing its finality. Hope and dread often arrive at the same moment.

"I pray for a call from the investigators, and then I dread the sound of the phone. Hope and fear live in the same room inside my chest." – P10

"When they say there are new findings, my heart runs forward and backward at the same time. Part of me wants the truth; the other part begs for one more day of doubt." – P2

Main Theme 2. Stories as Carriers of Grief and Memory

Stories kept loved ones present and protected their identities from erasure. Speaking became a way to carry grief together.

"We make his jokes at dinner time so that he can keep laughing. Time cannot wear out our photographs; they are the only photographs we have" – P8

"When I mention my sister, she sits beside me a little bit. Words become the thread that sews her back into our family." – P5

Subtheme 2.1. Testimony as survival

Giving testimony – formal or informal – was described as both painful and sustaining. Many saw it as an act of love and resistance.

"Giving my statement was like handing a candle to the darkness. It didn't bring him back, but it pushed the night a little further away." – P4

"I keep repeating the details to whoever will listen, not to hurt myself but to honor him. Should I keep silent, they will have taken even his name." – P7

Subtheme 2.2. Intergenerational transmission

Young people were taught history in family legends which were bitter and sweet. These stories formed identity and accountability.

"My daughter did not meet her grandmother, but she knows her favorite song. She drags this sorrow as a family heirloom and polishes it with her little hands." – P1

"We do not just simply teach the children what was done to us but rather who we are before it happened to us. I would have them succeed to bravery, and not to melancholy." – P9

Main Theme 3. Emotional Landscape of Exhumation

Exhumation days arouse strong, mixed feelings. Technical processes came into collision with individual memory and yearning.

"The day they began to dig, we were all anxious. It was like prying up an ancient sore, it was like letting out of the air at last." – P6

"I was a witness and a daughter by the site. My tears needed to wait behind the rules and the tape." – P2

Subtheme 3.1. Anticipation and anxiety

The anxiety of waiting until excavation and identification caused it to be impossible to calm down with routines.

"They explained the procedures, the boxes, the numbers, the DNA, and I nodded, but my heart heard only my father's name. Every step forward felt like a step toward a cliff." – P3

"I ironed my scarf three times before leaving for the site because my hands needed something to do. Anxiety made the hours stretch like years." – P7

Subtheme 3.2. Trauma triggers

Small objects and sensory cues unlocked old memories. The past returned without asking for permission.

"A single shoe in the dirt pulled me back decades in a breath. The smell of the earth became the smell of that day again." – P9

"I thought I had learned how to carry my grief, and then I saw a small button that looked like my sister's. The past rushed through me like cold water." – P5

Main Theme 4. Coping and Support Systems

Individuals relied on the family, neighbors, religion, and habit of seeing through the day. These unofficial supports in most cases substituted official care.

"On the hardest nights, my brother sits beside me, and we count our blessings until the list steadies us. Community is the blanket we wrap around the cold." – P4

"My prayers are quieter now, but they hold me together like stitches. When my hope depletes, I borrow hope from the neighbors." – P6

Subtheme 4.1. Informal resilience

Endurance was shaped through common meals, visits and prayers. The advice of the older ones made pain seek a spot to rest.

"After the investigators leave, the women gather to cook and talk, and somehow the pot of tea carries what our hearts cannot. We lean on each other the way walls lean to make a house." – P10

"The words of our elders do not erase the pain, but they give it a place to sit. Faith and family keep the floor under my feet." – P3

Subtheme 4.2. Barriers to psychological care

Seeking help was limited by distance, cost, stigma, and uncertainty about where to go. Many kept their suffering private to avoid judgment.

"People whisper that only 'mad' people see a counselor, so I kept my sadness at home. No one told us where to go, even if we wanted help." – P7

"The clinic is far, and the waiting room feels like a public confession. Sometimes it seems easier to be silent than to be seen." – P2

Main Theme 5. Healing Through Recognition and Justice

Naming, honoring, and accountability were considered as being on the way to healing. They were actually unable to reverse the loss but to restore dignity.

"She had a sense that the world was looking at her when they used her name in the ceremony. Dignity is a kind of medicine." – P1

"Justice will not stitch my heart back together, but it will stop the tearing. We need the truth to stand in the open, not hide underground." – P5

Subtheme 5.1. Rituals and reburial as closure

Rituals translated grief into collective action and offered a place to say goodbye. Many found a sense of movement after long stasis.

"We washed the dust from his bones with our tears and prayers, and for the first time I felt time moving again. The grave became a place to speak, not only to cry." – P4

"Carrying the remains to the cemetery gave weight to our love. The ritual taught my hands what my heart needed to do." – P8

Subtheme 5.2. Seeking acknowledgment and accountability

Formal identification and effects were put in terms of respect coming into view. Records, apologies, and prosecutions mattered for the living and the dead.

"I want an apology with a name and a signature, not just words blown away by the wind. Let them write our truth in the records so our children can read it." – P6

"Holding those responsible will not bring my mother back, but it will tell the world that her life had value. We deserve more than silence; we deserve answers." – P9

Discussion

Sorya map findings are easily matched to a recently extensive literature on ambiguous loss, memory work, and psychosocial aspects of forensic exhumation and transitional justice, and, in a way, provide context-specific details that add nuances to existing arguments. The subtheme, which is grief

existing "in limbo" between suspended habits and the unpredictability of relationships, corresponds with the ambiguous loss model that points out the possibility to have a form of "frozen grief" where the lack of structure prevents the closure (Boss, 2010, 2016). The descriptions of reluctance to use past-tense forms of the verb "to be" to reflect the missing and the daily switches between hope and fear are partial repeats of what Boss (2010) refers to as acute stress of unresolved absence. They are also reminiscent of needs recorded among families of the disappeared in Nepal, faced with everyday life between rumor and hope in the face of definitive knowledge (Robins, 2011).

The theme of "stories as carriers of grief and memory" complements ethnographic and transitional justice scholarship that documents how narration preserves personhood, sustains social bonds, and resists erasure. Riano-Alcala and Baines (2011) demonstrate how communities in rural Colombia organize testimony to produce living archives to re-write the missing into the social world. Similarly, Ferrándiz (2013) describes in Spain how talk, photographs, and collective remembrance practices around mass graves serve both as acts of love and as political claims to visibility. The Sorya subthemes add important nuance: testimony as both survival and endurance ("handing a candle to the darkness") parallels evidence that speaking out can be empowering, yet it is not uncomplicated; it can be painful and ambivalent, as also shown in research on truth-telling processes (Brounéus, 2010). The intergenerational transmission theme – teaching children "who we are before it happened to us" – resonates with literature on the interweaving of kinship, memory, and identity in contexts of disappearance, including the ways younger generations take up care for the dead and the missing to shape ethical and civic subjectivities (Schwartz-Marin & Cruz-Santiago, 2016).

"Emotional landscape of exhumation" sits at the intersection of technical procedure and intimate longing. The Sorya data capture the simultaneous "prying up an ancient sore" and "letting out air at last", which neatly illustrates the ambivalence reported across settings: exhumations can re-open wounds even as they enable mourning to proceed (Ferrándiz, 2013). Crossland (2013) emphasizes the resources of evidential regimes (boxes, numbers, DNA) in order to introduce specific temporalities and bureaucratic rationales which are not necessarily consistent with familial gaps of grieving and remembrance. Sorya's narratives vividly document this friction: procedural explanations "land" at an affective register saturated by loved ones' names. The theme of trauma triggers elicited by small objects is consistent with ethnographies of exhumation in Spain and elsewhere, where "witnessing objects" (buttons, shoes, fragments) act as conduits for sudden returns of the past (Ferrándiz, 2013; Crossland, 2013). The texts also illuminate the gendered labor of waiting and witnessing – women ironing scarves, organizing food, and holding vigil – echoing observations from Latin America and Mexico about the pivotal role of women's affective and organizational work in both remembrance and forensic processes (Schwartz-Marin & Cruz-Santiago, 2016).

This interpretation of "coping and supportive systems" intersects the public mental health studies within the humanitarian context: informal networks family, neighbors, religious practice tend to experience the greatest psychosocial burden in the context of gaps in formal care provision, logistical challenges, and stigma (Ventevogel et al., 2015). The Sorya stories of borrowing hope from neighbors and longing for the elders to impart pain and items to sit are consistent with findings that show that collective routines and shared belonging stiffens setbacks against communities with conflict (Miller & Rasmussen, 2010). Barriers to psychological care described here – distance, cost, uncertainty about services, and fear of being labeled "mad" – are typical of conflict-affected health systems and stigma-laden help-seeking environments (Ventevogel et al., 2015). Taken together, these results reinforce calls for integrated, community-based psychosocial support that is culturally anchored, low-threshold, and co-located with legal and forensic services (Ventevogel et al., 2015).

Finally, "healing through recognition and justice"

is strikingly consistent with scholarship that treats naming, identification, and dignified ritual as pathways to restore social and moral order, even when they cannot "reverse the loss". Families' desire for formal recognition – "an apology with a name and a signature" – and accurate records mirrors findings from Nepal, Spain, and the Balkans that emphasize the importance of documentation, acknowledgment, and transparency in official processes (Robins, 2011; Ferrándiz, 2013; Kovras & Robins, 2016). The Sorya accounts of ritual and reburial as movement out of stasis echo observations in Spain that exhumations catalyze "emotional communities" and enable grief to be translated into public, dignified action (Ferrándiz, 2013). At the same time, these data reiterate caution from elsewhere: participation in truth and justice processes can both support and strain survivors' well-being, requiring careful ethical and psychosocial accompaniment (Brounéus, 2010). The requirement that accountability should make the world know that her life was well-valued is consistent with research in Cyprus and Lebanon establishing that procedural transparency and convincing and family-centered investigations result in trust, and that obscurity raises injury and political estrangement (Kovras & Robins, 2016).

Two cross-cutting comparative insights emerge. First, Sorya's articulation of "waiting" as a social rhythm – lived through domestic routines, rumor ecologies, and the choreography of exhumation days – adds fine-grained texture to ambiguous loss literature, illustrating how temporal suspension is both an inner state and a collective practice (Boss, 2016; Robins, 2011). Second, the narratives foreground the moral economy of care that surrounds forensic work: from neighbors' tea to religious prayer, these "micro-practices" of support mediate the encounter between families and expert systems (Crossland, 2013; Schwartz-Marin & Cruz-Santiago, 2016). Compared to cases where robust victim-centered protocols have been institutionalized, the Sorya material suggests a heavier compensatory role for community supports in the face of limited formal psychosocial services and perceived stigma (Ventevogel et al., 2015).

Critically, the results trouble any linear equation of exhumation with healing. In line with previous research, exhumation and identification seem to be necessary but not sufficient to psychosocial relief; their beneficial action relies on clear process, a respectful approach, and inherent psychosocial accompaniment (Ferrándiz, 2013; Kovras & Robins, 2016). Similarly, testimony is resistance and risk: it has the potential to reclaim voice and presence, but it also has the potential to re-open wounds on the absence of proper support (Brouneus, 2010). Policy implications of the Sorya results support the following recommendations: incorporating culturally resonant ritual into forensic practice; ensuring that communication and transparency are family-centered; logistical and stigma obstacles to care are reduced with a community-based, low-intensity psychosocial intervention; and identify and rebury the dead with documentation, apology, and credible accountability.

Conclusion

Families of victims found in mass graves of Sorya live in a situation of suspended grief in between hope, memory and justice. Exhumation provides a form of recognition but no automatic curing; only when the forensic procedure is clearly defined and culturally located and assisted by the community-based care provides dignity and relief. It is important to include rituals, documentation, and psychosocial accompaniment in order to respect the dead and enhance the resilience of survivors.

Implications

The paper has indicated that forensic identification does not in itself solve grief. Families of mass grave victims need culturally sensitive rituals, open communication, and available community-based psychosocial support to influence successful healing. These aspects can be integrated in forensic and justice procedures to restore dignity, create resilience and eliminate stigma in conflict-impacted populations.

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Genetic Diversity in Hypervariable Segment I of Mitochondrial DNA Among the Kurdish Population Erbil City- Iraqi Kurdistan Region

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Abstract

Analysis of human sequence polymorphisms of mitochondrial DNA (mtDNA) hypervariable segment 1 (HV1) has become a significant technique in forensic casework and is used for genetic diversity and human evolution evaluation, consanguinity identification, and mitochondrial illness diagnosis. The present study aimed to provide insights into the demographic history and origins of the Kurdish people in the Iraqi Kurdistan region by analyzing mitochondrial DNA (mtDNA) sequence variations, particularly focusing on hypervariable segment 1 (HVS I). The HVS I of mtDNA was sequenced in 120 unrelated individuals from the Kurdish population by using Sangar sequencing. The study›s findings reveal significant genetic diversity among the Kurdish population›s HVS I of human mitochondrial DNA (mtDNA). The sequence of HVS I within the non-coding region had 413 mutations across 402 sites, resulting in 111 different haplotypes, as compared to the revised Cambridge Reference Sequence (rCRS) to gauge genetic diversity and community variety. Comparing this population sample to rCRS, the mutations found were (17.055%) transition and (0.921%) transversion. A considerable degree of community variety was revealed by the phylogenetic tree for the Kurdistan region. In order to determine the Kurdish ancestry and demographic history in the territory of Iraqi Kurdistan, the results also showed that the mtDNA haplotypes were classified into mtDNA haplogroups. The most frequent haplogroups were U, H, J, and HV, with frequencies of 22.5%, 19.2%, 14.5%, and 12.9%, respectively; nevertheless, T and N had a moderate prevalence of 8% and 6.4%, respectively. The haplotype results exhibited that Europe and India had the most genetic diversity in common with the Kurdish people of Iraqi Kurdistan. The findings will contribute to a better knowledge of Kurdish mitochondrial genome diversity, as well as to demographic history and forensic science.

Keywords: mtDNA, HVSI, Haplogroup Prediction, Genetic Diversity, Population Origin

Introduction

Mitochondrial DNA (mtDNA) analysis has emerged as a crucial technique in population genetics, anthropology, and forensic research (Sultana and Sultan, 2018). Due to its distinctive features, including maternal inheritance, high mutation rate, and lack of recombination, mtDNA functions as a potent molecular marker for examining genetic diversity, human migratory patterns, and ancestry (Kivisild, 2015). One of the most informative regions of mtDNA is the hypervariable region 1 (HVR1), which is located in the non-coding displacement loop (D-loop). This region rapidly acquires mutations across generations, rendering it especially valuable for tracing maternal lineages and assessing genetic links among populations (Johnson, 2013).

The HVR region is a part of the mtDNA D-loop,

and mitochondrial function depends on polymorphisms in the non-coding D-loop region (Kwaśniewski et al., 2023). The elevated mutation rate characteristic of these hypervariable regions serves a significant purpose in the fields of population genetics and human identification assessments. The current analysis focuses on specific hypervariable segments of mtDNA, namely HVI (nucleotide positions 16024-16365), HVII (nucleotide positions 73-340), and HVIII (nucleotide positions 438-576), which were evaluated to enhance the understanding of genetic diversity within the population (Verma et al., 2018). mtDNA haplogroups facilitate the classification of HVR1 and HVR2 haplotypes into associated categories. The high-throughput sequencing of mtDNA from distinct human lineages has been made possible by the advent of new molecular biology methods. These developments have proven useful in tracing the matrilineal descent of modern humans from Africa, who were afterward distributed to Asia and Europe (Kapoor et al., 2017).

The Kurdish people represent one of the largest ethnic groups in the Middle East without a sovereign state. Despite their significant presence in countries such as Iraq, Iran, Turkey, and Syria, the genetic background of the Kurdish population remains underexplored. Given the complex migratory history and cultural diversity of the region, investigating the mitochondrial genome of the Kurdish people can provide new insights into their maternal lineage, genetic affiliations, and evolutionary background.

Overall, this study analyzed the genetic diversity of the Kurdish population in Iraqi Kurdistan using mtDNA sequences from 120 unrelated individuals. Results showed a high level of genetic diversity, with 413 mutations and 111 distinct haplotypes. The most common haplogroups were U, H, J, and HV, with significant genetic similarity to Europe and India. This research contributes to understanding the Kurdish population›s genetic diversity and maternal ancestry and has implications for forensic investigations and anthropological studies.

Materials and Methods

Population Sample

For the current investigation, blood samples were taken from 120 unrelated Kurdish individuals from Erbil city, Iraqi Kurdistan region. All donors have received informed consent and information about their ethnic background. The study included only native peoples.

HVSI amplification via PCR and genomic DNA extraction

The genomic DNA was extracted from the blood sample via the quick protocol of AddPrep Genomic DNA Extraction Kit (Cat. No. 10023, KOREA). The DNA extraction was conducted in the laboratory of IMMUNOGENE CENTER, Erbil – Iraqi Kurdistan region. Since there are multiple copies of mt-DNA in every cell, a simple method to extract and measure DNA was used to amplify HV1.

According to a study performed by Tsutsumi et al. (Tsutsumi et al., 2004), amplification from 15,997 nt to 16,401nt of HVSI was done by using (forward primer: 5'CACCATTAGCACCCAAAGCT-3 and reverse primer:

5'TGATTTCACGGAGGATGGTG-3). In this experiment, these primers were used by applying a Polymerase Chain Reaction (PCR) machine to amplify the HVS1 in the mtDNA control region. The primers were validated in silico against the human reference genome through the application of a Basic Local Alignment Search Tool (BLAST) utilizing the 2.2.27 release number. The PCR conditions were established for the HVSI region, consisting of an initial denaturation step at 94 degrees Celsius for one minute, followed by a denaturation step at 94 °C for 1 min, and an annealing step at 60 degrees Celsius for one minute, and an extension step at 72 degrees Celsius for one minute. A final extension was conducted at 72 degrees Celsius for ten minutes. The thermocyclers were programmed for a total of 40 cycles. Subsequently, the PCR products were sequenced at the IMMUNOGENE CENTER utilizing the 3130

Genetic Analyzer (Applied Biosystems, Hitachi High Technologies, Tokyo, Japan).

Phylogenetic and statistical analysis

Multiple alignment

The electropherograms were read by the tool of BioEdit 7.1 (Hall, 1999). All sequences were aligned with the revised rCRS using the MUSCLE tool of MEGA 11 (Kumar et al., 2008). A phylogenetic tree was constructed to analyze the mtDNA haplogroups by PhyloTree 16 (Van Oven and Kayser, 2009). The error rate during haplogroup organization was lowered by implementing an algorithm in HaploGrep (Kloss-Brandstätter et al., 2011).

Haplogroup interpretation

MitoTool, a complimentary online bioinformatics application for examining mtDNA sequences (Ruiz-Pesini et al., 2007, Fan and Yao, 2011), was utilized to present the haplogroup results. MitoTool Clustal W was employed to align HVS1 sequences with rCRS and to identify the variations for each batch mode sequence. The precise coordination, whether optimal or close, governed the haplogroup via mt-DNA, considering its unique variation patterns. However, We independently verified the induced haplogroups using MitoTool›s mthap software. Ultimately, we integrated the MitoTool and mthap categorization data, and our phylogenetic tree has been developed to address and reconcile the inconsistencies in the derived haplogroups (Johnson et al., 2015). Finally, phylogenetically described haplogroups or branches were employed to assign individuals a potential haplogroup when MitoTool characterization and mthap yielded inconclusive or ambiguous results (more than one probable haplogroup per individual).

Statistical analysis

The evaluation of genetic diversity metrics and molecular variance analysis was performed using many criteria, specifically with Arlequin 3.5, as described by Excoffier and Lischer (Excoffier and Lischer, 2010). Moreover, various neutrality tests were implemented, including Tajima›s D (Tajima, 1989), Fu›s Fs (Fu, 1997), and R2 (Bektas et al., 2020). Furthermore, the mismatch distribution, as articulated by Harpending (Harpending, 1994), was assessed using two separate approaches that emphasize genuine demographic analysis. Finally, to determine and analyze the normal distribution data pertaining to abrupt population increases, the sum of squared deviations (SSDs) and Harpending›s raggedness score were utilized.

Results and Discussions

Sequence Diversities and Neutrality Test Statistics of HVS1

The use of mitochondrial markers in forensic casework requires knowledge of the prevalence of a particular mtDNA haplotype in a given population. The nucleotide sequences of the mtDNA D-loop (nt 15997-16401) and HV1 were determined in this study in a total of 120 unrelated people from the Kurdish-native speakers in the Iraqi Kurdistan region. All samples had PCR products with a size of roughly 420 bps, excluding the primers (Anderson et al., 1981, Andrews et al., 1999). As shown in (Table 1), which provides a statistical overview of the genetic diversity of the Kurdish population, with 111 distinct haplotypes detected among 120 individuals, overall diversity is fairly high. A grand number of 247 polymorphic sites were discovered in the Kurdish data collection. The low value of Tajima›s D (-1.76378) suggests that the population is growing.

Moreover, Iraq (-25.27102) (Al-Zahery et al., 2011), Iran (-25.30270) (Nasidze et al., 2006), Turkey (-25.06957) (Mergen, 2004), Kuwait (-25.40242) (Theyab, 2010) are the four countries having populations with negative Fs values, which suggest population growth. The findings of the mtDNA diversity and neutrality measures for the Kurdish population are shown in (Table 2). The genetic diversity measure shows that the Kurdish population (0.9986) is similar to nearby populations in Iraq (0.9918) and Saudi Arabia (0.9905), Turkey

(0.9851), Iran (0.9895), Syria (0.9881), Kuwait (0.9863), and Kurdistan/Iraq as well as kurmanje-T, Zazaki-T, (0.988), and (0.986). The Kurdish population›s Tajima›s D (-1.76378) and Fu›s FS (-96.565) values are significant at P>0.05 and P>0.005, respectively. These results suggest that the Kurdish population may be seeing growth in a relatively short period of time.

Table 1. Shows mitochondrial DNA HV1 sequence variability among the Kurdish population.

Genetic features	**Statistical data**
No. of the samples	120
No. of haplotypes (h)	111
No. of variable sites (s)	247
Haplotype diversity (S.D)	0.9986 (0.0013)
Nucleotide diversity	0.06901 (0.00855)
Average no. of nucleotide difference (K)	24.912
Tajima›s D	-1.76378
Fu›s FS	-96.565
Total no. of sites	402
Invariable (monomorphic) sites	87
Variable (polymorphic) sites	274
Total no. of mutation	413
Singleton variable sites	49
Parsimony informative sites	225

Table 2. Shows the mtDNA HVS1 sequence data and summary statistics for the Kurdish population, include the number of samples (N), gene diversity (H), nucleotide diversity, and Tajima's and Fu's FS.

Population	No.	Haplotype diversity	Nucleotide diversity	Tajima›s diversity	Fu›s FS	Reference
Kurdish	120	0.9986(0.0013)	0.06901(0.00855)	-1.76378	-96.565	Current study
Iraq	116	0.9918(0.0036)	0.019862(0.010659	−2.10200	−25.27102	(Al-Zahery et al., 2011)
Iran	92	0.9895(0.0049)	0.020453(0.010967)	−2.10033	−25.30270	(Nasidze et al., 2006)
Turkey	290	0.9851(0.0039)	0.017378(0.009419)	−2.22379	−25.06957	(Mergen, 2004)
Syria	69	0.9881(0.007)	0.019229(0.010419)	−2.14505	−25.44560	(Richards et al., 2000)
England	242	0.9651(0.0078)	0.014654(0.008122)	−2.24347	−25.48480	(Piercy et al., 1993)
Saudi Arabia	15	0.9905(0.0281)	0.022234(0.012566)	−1.32083	−7.63765	(Abu-Amero et al., 2007)
Kuwait	94	0.9863(0.0051)	0.0239(0.012661)	−1.87839	−25.40242	(Theyab, 2010)
Kurmanje -t	51	0.988		-2.05		(Nasidze et al., 2005)
Zazaki -t	27	0.986		-1.81		(Nasidze et al., 2005)

Haplogroup Predication of HVS1

According to a study conducted in Iraq by Al-Rashedi and colleagues, Arabs constitute approximately 75–80% of the Iraqi population, while Kurds, Turkmen, and other minority groups make up the remaining 20–25% (Al-Rashedi et al., 2015). Due to the prevalence of unique genetic sequences among different populations, each ethnic group possesses a distinct genetic structure.

mtDNA haplogroups-such as U, H, and J-are key maternal lineage groups that represent branches of ancient human ancestry. These haplogroups originated at various times and locations across the globe and are characterized by unique genetic markers (Kristjansson et al., 2023). Haplogroup U, for instance, emerged around 55,000 years ago, likely in the Middle East or Central Asia, and is now widespread across Europe, Western Asia, and North Africa. Its subclades, including U2, U4, and U5, are especially significant, with U5 representing one of the earliest mtDNA lineages found among indigenous European populations (Sahakyan et al., 2017).

Haplogroup H is believed to have originated between 20,000 and 25,000 years ago in Southwest Asia or Europe. Today, it is the most common mtDNA haplogroup in Europe, comprising 40–50% of the population, and is also present in North Africa and the Middle East (Sahakyan et al., 2017). Similarly, haplogroup J is uniformly distributed across Europe, with notable frequencies in regions such as Cornwall (20%), Wales (15%), and Iceland (14%), and is also common in the Middle East-particularly in Saudi Arabia (21%), Kuwait (16%), Kurdistan (15%), and Iraq (13%) (Dato et al., 2004, Clark et al., 2011).

The Kurdish population in Iraq exhibits a diverse mixture of mitochondrial haplogroups derived from European, Asian, and Indian ancestries, as shown by sample sequence analyses from MITOMAP (http://www.mitomap.org/). According to the data presented in (Tables 2 and 3), haplogroup U is the most prevalent among Kurds (22%), followed by H (19.2%) and J. Other haplogroups, such as N, R, L, M, K, and W, occur at lower frequencies within the Erbil population. The presence of these haplogroups suggests maternal genetic influences from both South Asia and Europe.

Supporting these findings, a study of various Kurdish subgroups- including Sorani, Hawrami, Kurmanji, and Kalhori- also demonstrated a high prevalence of haplogroups U, H, and J (Zarei and Rajabi-Maham, 2016). This genetic pattern implies significant recent gene flow from Europe into the Kurdish population. Notably, haplogroup U, which has an estimated age of 51,000 - 67,000 years, is substantially older than other haplogroups and appears widely across Europe and the Near East (Richards et al., 2000, Roostalu et al., 2007). Additionally, prehistoric European remains dating back up to 13,400 years have been linked to haplogroup U, further supporting its ancient presence in the region.

Comparative analyses of mtDNA haplogroup frequencies between the Kurdish population and neighboring populations revealed similar genetic patterns. As shown in (Table 4), the Kurdish population's haplogroup frequencies closely resemble those of populations in Iran and Iraq. Similar frequencies were also observed in Anatolia (Turkey), Syria, Saudi Arabia, and Kuwait (Triki-Fendri et al., 2016).

Moreover, our findings indicated a low presence of the African haplogroup L in Iraqi Kurdistan (3.3%) and Iran (2.2%), in contrast to a significantly higher frequency in Saudi Arabia (10.5%). The occurrence of haplogroup L in Iraqi Kurdistan points to historical African gene flow into the Kurdish population. Additionally, the presence of haplogroups M, N, and R in the Kurdish population serves as markers of gene flow from Asian regions, as demonstrated in (Table 4) (Figure 1).

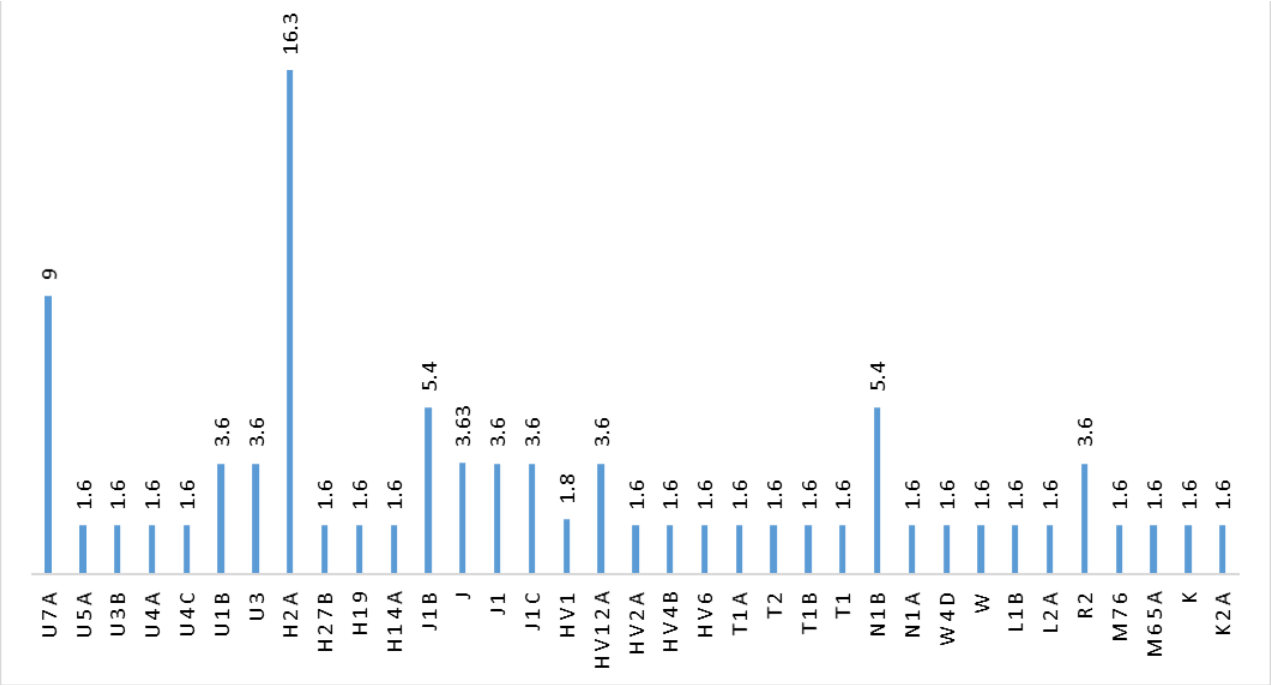
Table 3. Shows the comparison of haplogroup frequencies in the Kurdish population with different Regions in Iraq.

Population region	Sample size	Haplogroups frequency %	Ref.										
		U	H	J	HV	T	N	R	L	M	K	W	
Iraqi Kurdistan	120	22.5	19.2	14.5	12.9	8	6.4	3.3	3.3	3.3	3.3	3.3	This study
Iraq-Kurd	15	26.64	6.66	20	-	13.32	-	13.32	-	6.66-	-	6.66	(Al-Zahery et al., 2013)
Sorani	79	10	35	10	5	-	15	10	-	-	-	-	(Zarei and Rajabi-Maham, 2016)
Hawrami	79	-	71.4	-	4.76	4.76	5.26	-	-	-	-	-	
Kurmanji	79	21.04	31.56	5.26	-	-	-	-	-	-	-	-	
Kalhori	79	15.78	31.56	10.52	5.26	10.52	-	5.26	-	-	-	10.52	
Iraq	100	18	14	10	10	7	7	-	1	9	2	-	(Al-Rashedi et al., 2015)
Thi-Qar	216	19	19.9	9.3	10.6	8.8	-	-	1.4	1.4	3.2	1.9	(Al-Zahery et al., 2013)
Iraq	153	15.3	16.9	8.06	6.4	-	-	-	4	8.1	12	8.87	(Azzawi et al., 2013)
Basrah	164	9	17	11	5	-	6	-	5	9	5	2	(Ohied and Al-Badran, 2020)

Table 4. Shows the comparison of Haplogroup frequencies in the Kurdish population with other Neighboring populations.

Population	Sample size	Haplogroups frequency %											
		U	H	J	HV	T	N	R	L	M	K	W	Ref.
Iraqi Kurdsi-atn	120	22.5	19.2	14.5	12.9	8	6.4	3.3	3.3	3.3	3.3	3.3	This study
Iraq	100	18	14	10	10	7	7	-	1	9	2	-	(Al-Rashedi et al., 2015)
Iran	451	21.5	17.1	13.5	5.5	8.4	6.1	-	2.2	-	7.5	2	(Kivisild et al., 2003)
Anatolia	388	19.3	25	10.9	3.6	11.9	1.8	-	0.3	4.4	5.9	3.9	(Tajima, 1989)
Syria	69	15.9	24.6	10.1	4.3	10.1	-	-	5.8	1.4	4.3	2.9	(Ohied and Al-Badran, 2020)
Saudi Arabia	389	10.5	12.9	20.8	3.6	4.6	2.6	-	10.5	-	3.6	1.8	(Kivisild et al., 2003)
Kuwait	117	12	9	-	5	-	9.8	-	1.6	7	-	-	(Theyab, 2013)

Figure 1. Shows the frequency of haplogroup subclade on HVSI of mtDNA in the Kurdish population.



Phylogenetic analysis

To assess the genetic diversity of the Kurdish population in comparison to the revised rCRS, a neighbor-joining phylogenetic tree was constructed using MEGA version 11. As illustrated in (Figure 2), the tree was generated based on mtDNA HVS1 sequences from all samples relative to the rCRS. The analysis revealed a noticeable genetic divergence between the Kurdish population and the rCRS, which may be attributed to population-specific mutations. These findings align with a recent study that also reported a genetic gap between the Kurdish and rCRS populations, potentially due to region-specific mutations observed in the Basrah population (Ohied and Al-Badran, 2020).

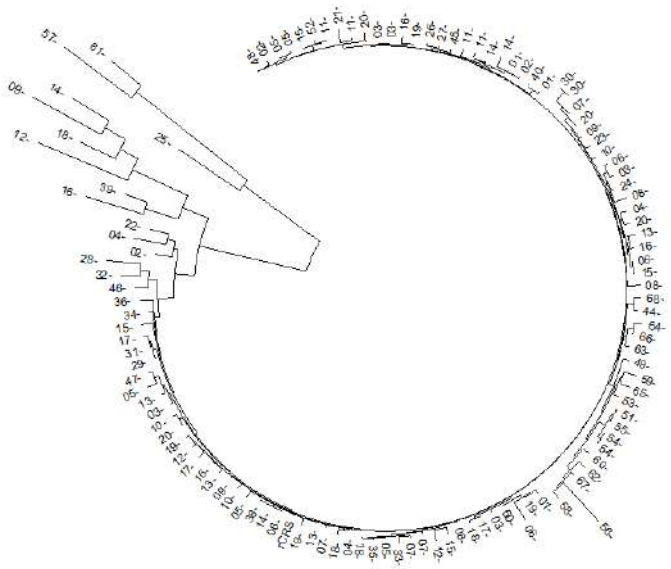


Figure 2. Shows the neighbor-joining tree of HV1 segment in Erbil/Kurdish population.

Sequence diversity of HV1

Table 5 summarizes all types of base mutations identified in the HVS1 of mtDNA, including both transitions and transversions. Among these, transition mutations were found to be the most prevalent. The most frequent transition was a thymine-to-cytosine (T→C) substitution, accounting for 11.170% of mutations, followed by a cytosine-to-thymine (C→T) transition at 3.217%, and a third, unspecified transition at 2.668%. In contrast, transversion mutations were comparatively rare, with a total frequency of only 0.921%. This observation aligns with previous literature, which consistently reports a predominance of transitions over transversions in human mtDNA variation (Hameed et al., 2015, Yang and Yoder, 1999).

Specifically, transitions were observed at positions 16100, 16122, and 16283, totaling 17.055%, while transversions were primarily detected at position 16292, accounting for the 0.921% frequency. Among all polymorphic sites, only two positions exhibited notably high levels of variation: position 16100, where the reference nucleotide T was substituted by C in 11.170% of cases, and position 16123, where a reference C was replaced by T in 3.217%. Despite these polymorphisms, the most frequent nucleotide at each site remained consistent with that of the revised rCRS. As shown in (Figure 3), the transition from T to C was the most frequent base substitution, comprising 62% of observed mutations, followed by C to T transitions at 18%. The least common mutation type was the A to T transversion, which constituted only 5% of the total.

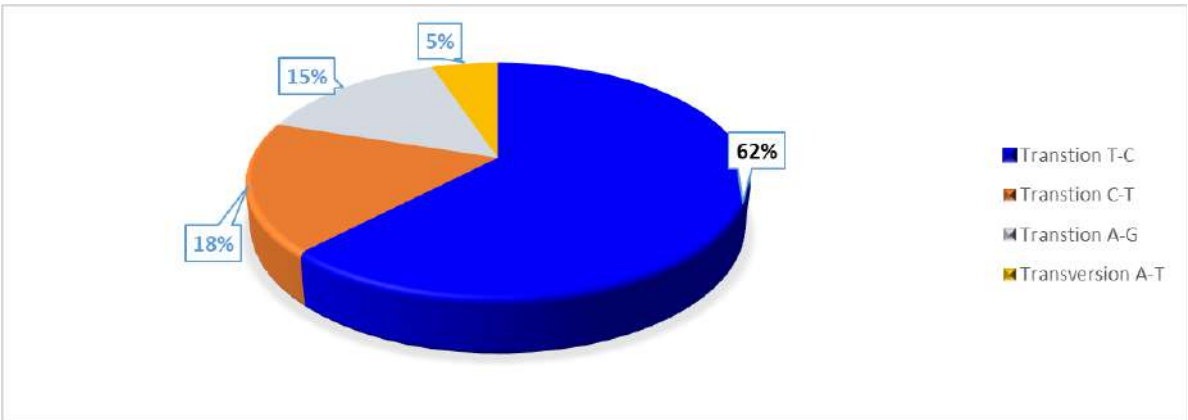
Previous studies have shown that the mtDNA coding region exhibits lower levels of genetic variability compared to the non-coding (regulatory) region (Walsh et al., 1991, Tang, 2002). To enhance the discriminatory power of forensic mtDNA analysis, the use of highly polymorphic sites is essential (Hadi et al., 2014). Particular attention should be given to selecting polymorphisms that are phenotypically neutral, especially within the mtDNA coding region, to avoid potential ethical concerns related to medical genetics and ethnic profiling.

Looking forward, comprehensive sequencing of the entire mitochondrial genome- including both coding and non-coding regions- combined with the use of informative single nucleotide polymorphisms (SNPs) from the regulatory region, may significantly improve the resolution and forensic utility of mtDNA profiling (Warshauer et al., 2013, Hadi et al., 2014).

Table 5. Type of base mutations on HVS1 of mDNA.

Mutation type	Base	Position	Frequency
Transition	T-C	16100	11.170%
Transition	C-T	16122	3.217%
Transition	A-G	16283	2.668%
Transversion	A-T	16292	0.921%

Figure 3. Shows the percentage of base mutation frequency on HVI of mtDNA.



Conclusion

The present study highlights the high level of mtDNA genetic diversity within the Kurdish population, as revealed by the analysis of the HVS1. The Tajima's D test yielded a value of -1.76378, suggesting an excess of low-frequency polymorphisms, which may be indicative of population expansion or purifying selection. Similarly, Fu's Fs statistic, with a strongly negative value of -96.565, further supports the presence of population growth and demographic expansion. The nucleotide diversity was calculated at 0.06901, while the haplotype diversity reached 0.9986, indicating a remarkably high level of genetic variability in the population.

In terms of haplogroup distribution, the Kurdish population is predominantly represented by haplogroups U (22.5%), H (19.2%), and J (14.5%). These maternal lineages are associated with ancient human migrations across Europe, the Middle East, and Central Asia, providing valuable insights into the historical and evolutionary origins of the Kurdish people.

The genetic patterns observed in this study not only support the complex maternal ancestry of the Kurdish population but also offer a deeper understanding of their historical demographic processes. The combination of high haplotype diversity and signals of past population expansion underscores the genetic richness and historical mobility of Kurdish-speaking groups.

However, despite these important findings, the study has certain limitations that should be acknowledged. The sample size of 120 individuals may not adequately represent the genetic diversity of the entire Kurdish population, and the focus on individuals from Erbil City limits the geographic scope, potentially excluding variation present in other Kurdish regions such as Sulaimani and Duhok. Moreover, the exclusive analysis of the HVS1 region, while informative, may overlook additional variations found in other mtDNA segments like HVS2 and HVS3. The study also does not explore correlations between mtDNA variants and clinical conditions, which could be essential for understanding mitochondrial disorders in this population.

Consequently, continued genetic analyses of mtDNA particularly when integrated with data on nuclear DNA, Y-chromosomal markers, and archaeological evidence can significantly contribute to reconstructing the migration history and ancestral origins of Kurdish populations. These findings open avenues for more comprehensive research into the genetic legacy of populations residing in the broader Middle Eastern region.

Abbreviations

BLAST	Basic Local Alignment Search Tool
HV1	Hypervariable segment 1
HVR I	Hypervariable region I
HVS I	Hypervariable site I
mtDNA	Mitochondrial DNA
PCR	Polymerase Chain Reaction
rCRS	Cambridge Reference Sequence
SSDs	Sum of squared deviations

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