CASE STUDY

Study on the Variants, of Mitochondrial HVa1 and HVa2 Regions in Iraqi Kearns-Sayre Syndrome: A Case Study

Monem M. Alshok,[1] Mona N. Al-Terehi,[2] Mohammed A. Jawad,[3] Ali H. Al-Saadi,[2] Abed J. Kadhim[3]

[1] *College of Medicine, Babylon University, Hillah, Iraq*
[2] *College of Science, Babylon University, Hillah, Iraq*
[3] *Al-Nisour University College, Baghdad, Iraq*

Received: 22nd July, 2021; Revised: 12nd August, 2021; Accepted: 01st September, 2021; Available Online: 25th September, 2021

ABSTRACT

Kearns-Sayre syndrome (KSS) is a rare syndrome characterized by mitochondrial myopathy. The present study was carried out to report an unusual manifestation of 18 years old Iraqi female patient suffering from KSS symptoms; blood samples were collected to estimate sequences of mitochondrial DNA for patients. The deoxyribonucleic acid (DNA) was extracted using kits then PCR-sequencing using to amplification HV1a and HV2a loci in mitochondrial DNA. The result shows that case sequences' identities with virtual amplification were 8.48% and 41.8%, while 95.53% and 80.06% were healthy Iraqi individuals for the HV1a and HV2a, respectively. The multiple comparisons show that there were more variants in HV2a than HV1a site, it was variant in 105 sits while in HV1a were 11 variants, we concluded that some variants which represented by a percentage of differed with healthy Iraqi individuals mtDNA 4.47% and 19.94% for the tow loci in the present study might be contributed in the phenotype of KSS case, but we need to investigate more site of mtDNA and genomic DNA.

Keywords: Kearns-Sayre syndrome, Mitochondrial DNA, Syndrome detection.

International Journal of Pharmaceutical Quality Assurance (2021); DOI: 10.25258/ijpqa.12.3.30

How to cite this article: Alshok MM, Al-Terehi MN, Jawad MA, Al-Saadi AH, Kadhim AJ. Study on the Variants, of Mitochondrial HVa1 and HVa2 Regions in Iraqi Kearns-Sayre Syndrome: A Case Study. International Journal of Pharmaceutical Quality Assurance. 2021;12(3):335-341.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

A syndrome known as Kearns-Sayre syndrome (KSS) is an uncommon disorder of multi-organs that affects below to 20 years of female and males age. Its main features are progressive external ophthalmoplegia, weakness in muscles, degeneration in retina, cardiac defects in the form of conduction block,[1] a range of hearing deficiencies, with different changes in the nervous system (central and peripheral) which characterized by elevated in the level of cerebrospinal fluid protein, cerebellar signs, destroy cognitive dysfunction, and endocrine manifestations including diabetes mellitus[2], short stature and other disorders in endocrine. Different types of mitochondrial encephalomyopathy of the nervous system included destroy in vestibular, optic atrophy, myopathy, poor intellectual development or mental deterioration, pyramidal signs, present apart from hypothyroidism, hypogonadism, hyperparathyroidism, and renal dysfunction. On the other hand, several factors contribute to their characteristic general appearances like poor musculature, frequent secondary associated musculoskeletal defects in kypho-scoliosis, hyperlordosis, frequent wasting, and typical facies. Investigators recorded partial deletions in mitochondrial DNA (mtDNA) in 1988 for KSS syndrome.[3-5]

The more severe syndromic variant is chronic progressive external ophthalmoplegia (CPEO), classified as a heterogeneous neuromuscular disease resulting from different types of mitochondrial DNA mutations. The phenotypic expression of these mutations is based on the number and system types. The disturbance of cardiac conduction is responsible for high mortality syndrome. The frequency of KSS appearances is 1.6 cases per 100,000 population.[6,7]

The first recorded of this syndrome patients by Thomas P. Kearns and George P. Sayre (1958) how investigated patients with pigmentary retinopathy, external ophthalmoplegia, and cardiac conduction block (CCB), at the next time it called KSS.[8-10]

The Maine causes of KSS have resulted from mitochondrial DNA (mtDNA) deletion leads to particular medical symptoms.[11] Mitochondrial DNA is composed of 16,569 bp, included 37 genes, thirteen genes encode to electron transport chain (ETC) proteins and 22 encode to transfer ribonucleic acid RNA (tRNA). The other two genes encode to subunits of ribosomal RNA (rRNA).[12] The Mutations in ETC lead to impaired

*Author for Correspondence: mohammed.a.medical.lab@nuc.edu.iq

energy produced by mitochondria. This is most readily in tissues dependent on aerobic metabolisms like the skeletal brain, cardiac muscles, and kidneys. It is considered one factor that contributed to mitochondrial diseases.[13] The size and loci of mutation have role in mitochondrial disease types and progressive, Mitochondria replicate with cell division during gestation and throughout life, thus any mutation would be inherited to a new cells or causes an uneven distribution of dysfunctional mitochondria within each cells which cause heteroplasmic responsible of diseases like KSS. The mutated mtDNA in each cell, tissue, and organ, is dependent on when and where the mutation has occurred.[14] The aim of this presentation, a case of an 18-year-old female patient with a clinical diagnosis of KSS with an associated external ophthalmoplegia, mild muscular weakness, mild sensorineural deafness with cardiac conduction defect, was reported as a rare case in the Iraqi population.

MATERIALS AND METHODS

Genetic Study

- Sample collection; about five mL of whole blood was collected from KSS case and control for DNA extraction, which performed the Favor gene extraction kit then concentration and purity were detected using nano drop.[15]
- Polymerase chain reaction (PCR) mtDNA was amplification in 2 sites using the following primers (Table 1)
- The condition of amplification by PCR and size products, PCR experiments implemented for all primers; 5 minutes of per-denaturation at 94˚C, then 35 cycles consist of (94˚C -30 sec, 58.4˚C - 30 sec, 72˚C - 30 sec) then 10 minutes at 72˚C for one cycle. The PCR products detection by electrophoresis pattern (1% agarose, 75 V, 20 mA for 40 minutes). The PCR size product of the first primer was 280 for HV1a (15978-16257 in *Homo sapiens* mitochondrion) and 278 for HV2a (29-306 *Homo sapiens* mitochondrion), data analysis by NCBI blast, https://blast.ncbi.nlm.nih.gov/Blast.cgi, virtual amplification performed by sms bioinformatics software http://www.bioinformatics.org/sms2/pcr_products.html, and multiple alignments using MAFFT version 7 https://mafft.cbrc.jp/alignment/server/.

RESULTS

Report of A Case

Hawra'a Suhail Kareem is a 18 years-old female patient. She's 4th child to healthy-consanguineous parents. There was unremarkable family history unless diabetes mellitus and hypertension of her father during recurrent pulmonary infection and upper respiratory tract allergy of her mother and one of her sisters. Till now, case had normal development of neuropsychomotor. There was no reference to neo- or perinatal intercurrents. Also, the case suffered from stature mild deficient growth begun at 13 years old of her age when she started to complain of progressive "dropping eyes" plus sight disturbances without diplopia, and progressive mild hearing loss also, however no unsteady gait or symptoms of cardiac failure, she complains of occasional exertional dyspnea. Other features were observed on a case, like mild progressive muscle weakness and generalized mild muscle wasting lead to a loss of more than 5 kg of body weight. During this time, she was unable to complete her primary school studies. Several months ago, she was admitted to the hospital due to symptoms of tiredness and severe headache plus arterial hypotension, and a brain CT scan showed suspicious intracranial calcifications. On examination in the medical ward, she has incomplete bilateral palpebral ptosis. The nails and teeth were normal. She has mild pigmentation of her skin with excessive growth on her face and extremities, in the case has very mild generalized muscle wasting with a weight of 53 kg and height of 153 cm (Figure 1).

The examination of the skin showed mild hyperpigmentation of the elbow region, increases of the hands and mucosal areas of her mouth. a mild diffuse enlargement of the thyroid gland was shown in the neck (Figure 1) with excessive growth on her chin and mustache area. Blood pressure was 95/65 mmHg. She was well orientated and didn't have language or speech problems, and was cooperative to answer the question; however, we noticed mild memory functions abnormalities, there wasn't any defect in Cranial nerves VII, IX, and X. Trousseau's and Chvosteck's signs were negative ophthalmological examination shows little retinal dystrophy with visual acuity of 7/10 (right) and 8/10 (left). CSF examination not done There is normal sodium, potassium, chloride, bicarbonate, calcium, levels in serum and low T4 and normal TSH, glucose: 5 mmol/L; mildly elevated in creatine phosphate were present, other

Table 1: Primers sequences used in mtDNA amplification sites.[16,17]

HV1a	A1 (L15997)	F- CAC CAT TAG CAC CCA AAG CT
B2 (H16237)		R- GGC TTT GGA GTT GCA GTT GAT
HV2a	C1 (L 048)	F- CTC ACG GGA GCT CTC CAT GC
D2 (H 285)		R-GGG GTT TGG TGG AAA TTT TTT G

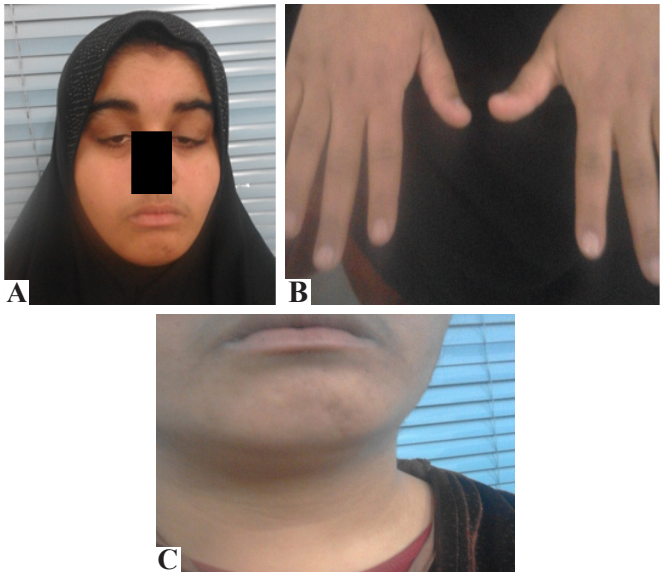

Figure 1: (A) Caseface (with permission of patient); (B) Mild skin pigmentation, No nail abnormalities; (C) Neck appearance, mild thyroid swelling.

testing were normal included (the serum phosphate, aspartate aminotransferase, albumin, alkaline phosphatase total protein, creatinine, and magnesium). Hyperphosphaturia, hyperaminoaciduria, and glucosuria didn't found in urinalysis. the skeletal was observed normal and audiometric estimation demonstrated mild bilateral sensorineural hearing loss. The Twelve-lead electrocardiography (ECG) appeared as a 2:1 atrioventricular (AV) block with slow ventricular rate. Intermittent complete AV block, and complete left bundle branch block (LBBB) at one time and ST, T waves changes seen on (Figure 2). Echocardiography showed mild left ventricular dysfunction and dilated coronary sinus (Figure 3), urine analysis showed proteinuria, no aminoaciduria. CXR and abdominal U/S were reported as normal; CT brain scan demonstrated mild intracranial calcification. Unfortunately, the patient was reluctant to have another MRI scan of the brain as her previous one was lost. We regularly follow the patient yearly.

Genetic Results

The genetic analysis of tow high variable regions show that the virtual amplifications of HV1a and HV2a were 280, 278 bp, respectively, the virtual amplification used in present report because there is the first study about mitochondrial DNA sequencing for Iraqi KSS as well as there is no previous data recorded about these loci sequencing in Iraqi population however there is a parallel study performed for using this loci for human identifier between Iraqi individuals in forensic applications (data not shown). Thus we used NCBI data for comparable with this case (Table 2, Figure 3A and B).

The identities of case sequences with stander control (virtual amplification) was 8.48% and 41.8% while it was 95.53% and 80.06% with healthy Iraqi individuals for the first and second loci, respectively (Figures 4 and 5). Mitochondriopathies can present with a large number of heterogeneous genotypic and phenotypic manifestations associated with several enzymatic defects and in the case reported we demonstrate the main defects in mitochondrial DNA.[14] The differed percentage with healthy Iraqi individuals was 4.47% and 19.94% for the tow loci, respectively, these differences may be contributed of phenotype in our case; it need more investigation for analysis another site in mtDNA and genomic DNA for detection to predict related genes with KSS in Iraqi population, also genetic predispositions and family history.

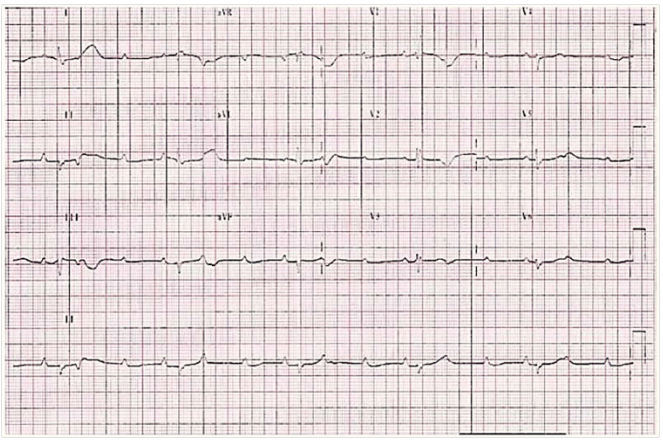

Figure 2: Echocardiography showed mild left ventricular dysfunction

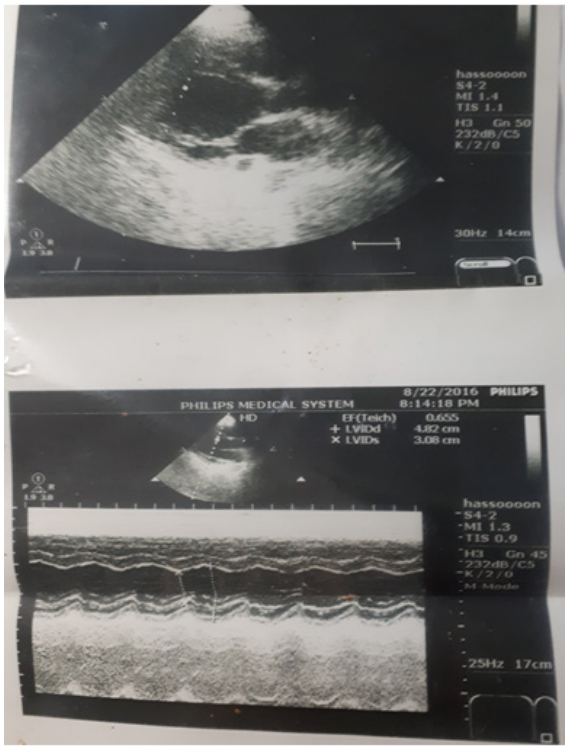

Figure 3: Cardiac 2D echo which showed dilated coronary sinus

Table 2: DNA sequences of virtual amplification of HV1a and HV2a with PCR size products.

Loci	*DNA sequence of virtual amplification*	*Size products*
HV1a	CACCATTAGCACCCAAAGCTAAGATTCTAATTTAAACTATTCTCTGTTCTTTCATGGGGAAGCAGATT TGGGTACCACCCAAGTATTGACTCACCCATCAACAACCGCTATGTATTTCGTACATTACTGCCAGCC ACCATGAATATTGTACGGTACCATAAATACTTGACCACCTGTAGTACATAAAAACCCAATCCACATC AAAACCCCCTCCCCATGCTTACAAGCAAGTACAGCAATCAACCCTCAACTATCACACATCAACTG CAACTCCAAAGCC	280
HV2a	CTCACGGGAGCTCTCCATGCATTTGGTATTTTCGTCTGGGGGGTATGCACGCGATAGCATTGC GAGACGCTGGAGCCGGAGCACCCTATGTCGCAGTATCTGTCTTTGATTCCTGCCTCATCCTATTA TTTATCGCACCTACGTTCAATATTACAGGCGAACATACTTACTAAAGTGTGT	278
	TAATTAATTAATGCTTGTAGGACATAATAATAACAATTGAATGTCTGCACAGCCACTTTCCACACA GACATCATAACAAAAAATTTCCACCAAACCCC	

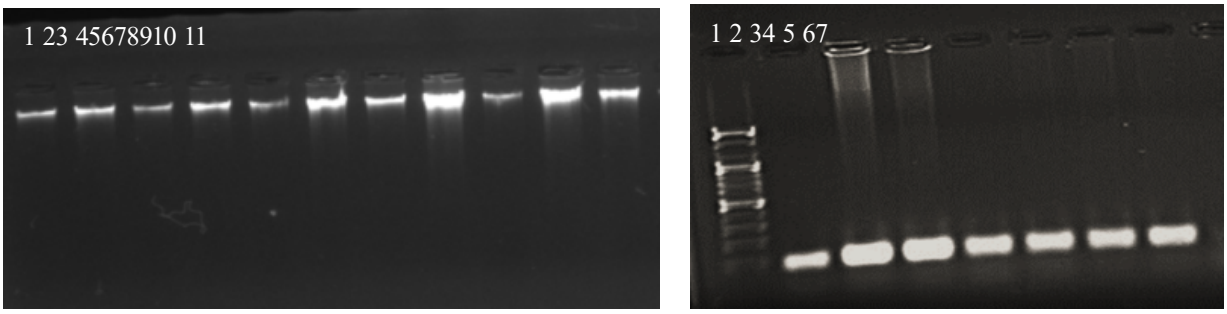

Figure 4: A) electrophoresis pattern of DNA extraction from case (lane 10) and control (lane1-9), B) electrophoresis pattern of PCR product of HV1a (above image) and HV2a (lower image) 1% agaose, 70 V and 20 mA for 60 minutes.

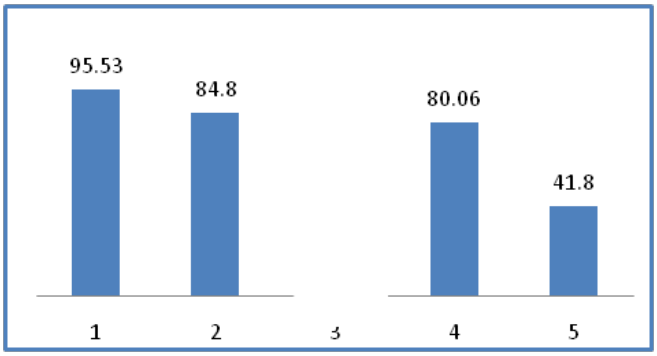

Figure 5: The percentage of identities between case and healthy Iraqi individuals, lane 1 and 2 HV1a between case and healthy Iraqi individuals, stander control, respectively; lane 4 and 5 HV2a between case and healthy Iraqi individuals, stander control.

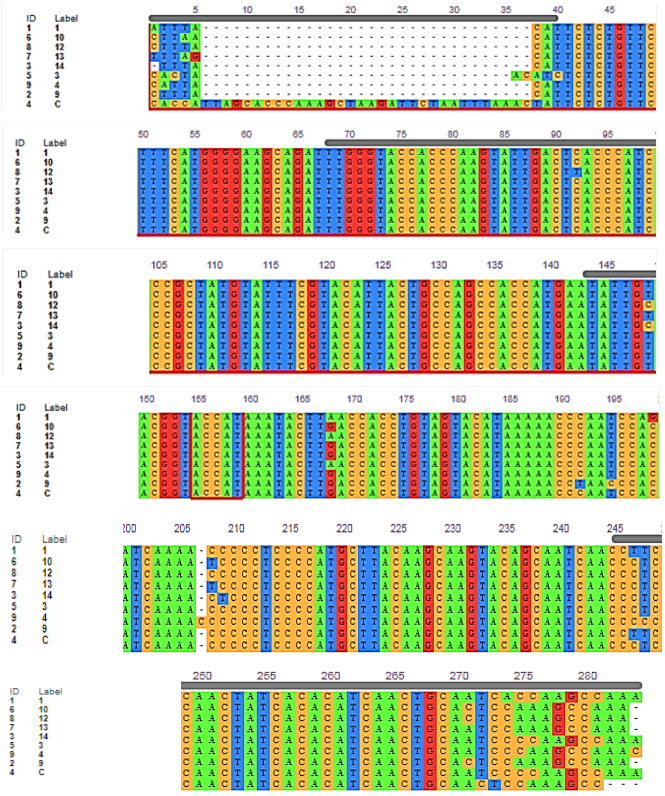

Figure 6: Multiple comparisons of mitochondrial DNAHV1a, lane 1 case sequences, lane 3,4,9,10,12,13,14 healthy Iraqi individuals sequence, C NCBI sequenceID: NC_012920.1.

Table 2: Variations in HV1a of KSS

Variant loci	*Types of variant C>K*	*Identities with Iraqi healthy individual (%)*
16009	C>A	71.42
16010	A>T	57.14
16011, 16012	C>T	85.71, 71.42
16014-16045	Deletion	100%
16046, 16257	T>C	85.17, 100
16292,16293,16294	Insertion	100, 28.57

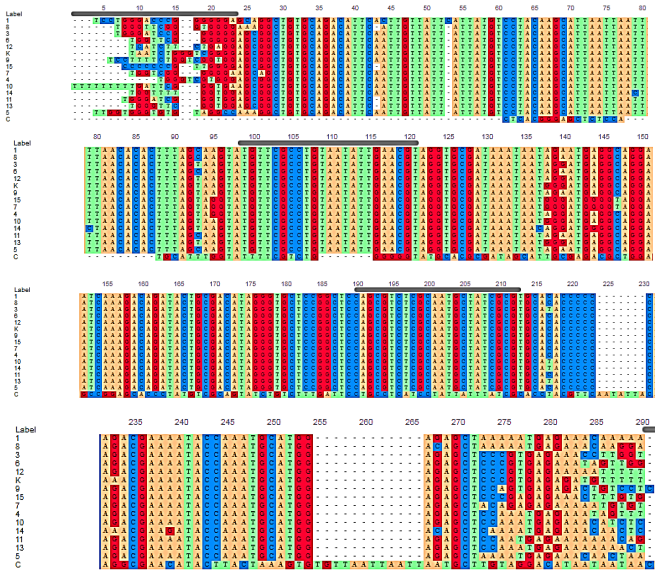

Figure 7: Multiple Comparisons of mitochondrial DNA HV2a, lane k case sequences, lane 12,3,6,9,15,7,4,5,1,8 healthy Iraqi individuals sequence, C NCBI sequence NC_012920.1 (29-306)

The results of multiple Comparisons of HVa1 are shown in Figure 6 and Table 3. It shows there were high identities with Iraqi individuals fewer identities with stander control there was differentiated in 11 loci, two were deletion, three were insertion and six were substitutions mutations.

The results of HV2a variable show high variation between patient and stander control while it shows high identity with healthy Iraqi individuals as shown in Figure 7 and Table 3. The results show 105 loci were differentiated with stander control 4 were insertion, three sites were deletion while others were substitution

Table 3: Variations in HV1a of Kearns-Sayre syndrome

Variant loci	*Types of variant C>K*	*Identities with healthy Iraqi individuals, respectively*
35-89, 107-116 138-144, 284-295	Insertion	Variable,100, 100, 100
91, 134, 195, 212, 216, 227, 246, 273, 302, 304	T>C	100, 100, 100, 100, 100, 100, 73.33, 100, 80, 26.66
92, 101, 104, 105, 119, 154,182, 242	C>T	100, 100, 100, 100, 100, 100, 100, 100
95,96, 99, 124, 146, 147, 170, 181,184,185, 202, 262, 263	G>A	100, 100, 80, 100,100, 100, 100, 100, 100, 100, 6.6, 100
98, 189, 201, 234, 238, 279	A>C	100%, 100, 100, 100, 100, 100
102, 158, 164, 188, 190, 191, 192, 200, 245, 268, 276	C>A	100, 100, 100, 100, 100, 100, 100, 100, 100, 93.33, 100
103, 123, 161, 177, 220, 229, 236, 274, 298	T>A	100,100, 100, 100, 100, 100, 100, 100, 100, 100
118, 159, 163, 196, 208, 281, 306	G>T	100, 100, 100, 100, 100, 100, 40
121, 129, 152, 167, 197, 205, 207, 209, 233, 239, 250, 251, 282, 311, 314	T>G	100, 100, 100, 26.66, 100, 100, 100, 100, 100, 100, 100, 100, 6.66, 0
148, 249, 303	G>C	100, 100, 80
155, 215, 243, 278, 297, 305	A>G	100, 100, 100, 100, 86.66, 40
169, 174, 206, 223, 228	C>G	53.33, 100, 100, 100
171, 226, 313, 315, 317, 319	A>T	100, 100, 6.66, 26.66, 40, 33
253-259, 320	DELETION	100, 100

DISCUSSION

Mitochondriopathies can present with a large number of different genotypic and phenotypic manifestations associated with several enzymatic defects.[18,19] And in the case reported, we demonstrate the main defects in mitochondrial DNA, in the form of EOP, and cardiovascular defect, a similar study[20] found that the mitochondrial DNA heteroplasmy unfolded protein response caused maintenance of deleterious mitochondrial DNA. A transcriptional response triggered by defects in oxidative phosphorylation such as observed in some KSS cases contributed to promoting the recovery and regeneration of defective mitochondria. About 4.9kB of mtDNA was deletion observed in third of KSS cases.

These mitochondrial diseases can present at different ages, including the case presented at early adolescent age, but the disease might remain unrecognized till adulthood[21] had been reported . The ophthalmopathy related to KSS and the hearing loss which were described in several other studies[21-24] and depending on their extent and severity, might have some prognostic points for disease progression, although some studies[24] mentioned that it has no rule in predictive value. Endocrinopathies are largely associated or related to KSS. Our patient has mild short stature, excessive facial hair growth. The defect might be related to pituitary growth hormone deficiency . The cardiac defects are related to variable degree of conduction system dysfunction associated with several mitochondrial disorders and considered the most important prognostic factor.[11,25-27] The well-known characteristic finding in KSS of Brain MRI scan consists of combining the high-signal foci in subcortical cerebral white matter and in the brain stem, globus pallidus, or thalamus.[28,29] Unfortunately, our patient refuse to do this test. On several occasions, the presenting patient complains of bilateral loins pain with dysuria, on urine testing fail to detect any abnormalities, though hypoparathyroidism and an associated renal tubular dysfunction has been recently described[30-32] in KSS. Currently, the patient on regular follow up and was given symptomatic treatment and folic acid 5 mg daily was prescribed, no specific treatment has been effective of KSS. The treatment is palliative and supportive for the clinical conditions. Cases have myopathy use of coenzyme Q10, especially in patient with mutations reduced synthesis of this protein.[33-38] The inheritance of the mitochondrial genome is maternally,[26] because of loss of paternal mitochondria with sperm tail during fertilization. Mitochondria are responsible of the production of intracellular ATP, which is necessary to supply energy for various metabolic functions. The most widely accepted diagnostic criteria in the literature are the triad: progressive external ophthalmoplegia, pigmentary retinopathy, and Complete Cardiac Block. By 1992, same researchers[30] characterized 226 cases of KSS. Remes *et al*.[39] estimated a prevalence of 1.6 cases per 100,000 in a Finnish population (6 patients, only 3 had clinical features for Kearns-Sayre syndrome). Schaefer *et al*.[40] detected a prevalence of 1.17 cases per 100,000 population of large-scale mitochondrial deletions in North East England.

The variation of the two loci DNA may be an association with the phenotype of KSS, which described in case report above; however other loci needed investigation to detection DNA state, present study improved that KSS has more variation in HV2a than HV1a, this is the first study deal with this mtDNA loci in Iraq also no previous studies about these loci, Ya *et al*., (2016) studied overlap MERRF/KSS syndrome they found that it associated with them. A3243G mutation and single large-scale mtDNA deletions (from 8470 to 13,446, totally 4977 bp).[41]

Also, present results deal with large-scale mitochondrial DNA (mtDNA) deletions that have been described among

patients who have a mitochondrial myopathy related to chronic progressive external ophthalmoplegia (PEO), including KSS.[42,43] Such deletions now are considered the hallmark of KSS. A point mutation in the transfer RNA genes (leucine in MELAS and KSS.[44]

A previous study showed that the number of the mitochondrial genome affected in patients of PEO were varying from 27% to 85%. Thus it differs in different tissues from the same patients as well.[45] This suggests that expression of mitochondrial disease may require a threshold number of mutated mtDNA.[46,47] The ophthalmoplegia invariable manifestation of mtDNA deletions has been unknown. It may be because the external ocular muscles were derived from a few embryonic cells with a large number of mutated mtDNA. The mitochondrial volume fraction in eye muscles is three to four times that of limb muscles.[48] The identical deletion may be correlated with both KSS and pure ocular myopathy, Moraes *et al*[46] improved that KSS is the full expression of a pleiotropic disorder. Other studies suggested that mitotic segregation may be causes differentiated in clinical phenotypes.[49] There is a random distribution of mitochondria into daughter cells during early cell division, causing some cells to retain a mixture of wild and mutant DNA (heteroplasia). Thus, the percentage of affected mitochondrial genome differs in various tissues from the same patients.[50, 51,49]

CONCLUSION

The KSS is a rare syndrome, and resources for diagnosis are not widely available; we should have significant clinical suspicion and genetic research for detection syndrome. We noticed that KSS prognosis is related to the number of organ system affected and the severity of the alterations. The disturbances in the cardiac conduction system are responsible for high morbidity and mortality of the disease.

REFERENCES

1. Foyaca-Sibat H, Ibańez-Valdê L. Kearns-Sayre Syndrome "Plus": A Case Report . The Internet Journal of Neurology. 2001;(1);2:103
2. Rolak LA. Kearns Sayre in Neurology Secrets 2nd Edition 1998 by Hanely & Belfus .Inc. :PP 57-59
3. Katsanos KH, Elisaf M, Bairaktari E, Tsianos EV. Severe hypomagnesemia and hypoparathyroidism in Kearns-Sayre syndrome. Am J Nephrol. 2001;21(2):150-153.
4. Pasternak JJ. An Introduction to Human Molecular Genetics: Mechanisms of Inherited Diseases. 2nd ed. New Jersey:Wisley. 2005.
5. http://www.ninds.nih.gov/disorders/Kearns-Sayre.htm
6. Nasseh IE, Tengan CH, Kiyomoto BH, Gabbai AA. Doenças mitocondriais. Rev Neurociências. 2001;9(2):60-69.
7. Wabbels B, Ali N, Kunz WS, Roggenkämper P, Kornblum C. Chronic progressive external ophthalmoplegia and Kearns-Sayre syndrome:interdisciplinary diagnosis and therapy, Ophthalmologe. 2008 Jun;105(6):550-6. doi: 10.1007/s00347-007-1643-5.
8. Kearns TP, Sayre GP. Retinitis pigmentosa, external ophthalmoplegia and complete heart block: unusual syndrome with histologic study in one of two cases. Arch Ophthalmol 1958;60:280-289.
9. Zago Filho LA, Shiokawa N. Sٍndrome de Kearns-Sayre: relato de dois casos. Arq Bras Oftalmol 2009;72(1):95-8.
10. Villacorta H, Catharina CV, Nogueira AS, Duarte MM, Roma CCC, *et al*. Bloqueio atrioventricular total em sٍndrome de Kearns-Sayre: relato de caso. Arq Bras Cardiol. 1996;67(2):107-
11. Tuppen HA, Blakely EL, Turnbull DM, Taylor RW. Mitochondrial DNA mutations and human disease. Biochimica et Biophysica Acta (BBA)-Bioenergetics. 2010 Feb 1;1797(2):113-128.
12. Shadel GS. Expression and maintenance of mitochondrial DNA: new insights into human disease pathology. The American journal of pathology. 2008 Jun 1;172(6):1445-1456. doi:10.2353/ajpath.2008.071163
13. Shadel GS. Expression and Maintenance of Mitochondrial DNA : New Insights into Human Disease Pathology. *The American Journal of Pathology*. 2008;172(6):1445-1456. doi:10.2353/ajpath.2008.071163.
14. Schon EA, DiMauro S, Hirano M. Human mitochondrial DNA: roles of inherited and somatic mutations. *Nature reviews Genetics*. 2012;13(12):878-890. doi:10.1038/nrg3275.
15. Al-Terehi1 M, Hasan A, AL-Jboory JM, Al-Saadi1 A, Zaidan H, Obiad S. Haplotype Polymorphisms in Cytokines Genes Using Pcr-Sscp Technique in Iraqi Breast Cancer Patients Der Pharma Chemica, 2016;8(22):27-31.
16. Bašić Ž, Fox AR, Anterić I, Jerković I, Polašek O, Anđelinović Š, Holland MM, Primorac D. Cultural inter-population differences do not reflect biological distances: an example of interdisciplinary analysis of populations from Eastern Adriatic coast. Croatian medical journal. 2015 Jun 15;56(3):230-238. doi:10.3325/cmj.2015.56.230
17. Hedman M, Brandstätter A, Pimenoff V, Sistonen P, Palo JU, Parson W, Sajantila A. Finnish mitochondrial DNA HVS-I and HVS-II population data. Forensic Science International. 2007 Oct 25;172(2-3):171-178. Epub 2007 Mar 2
18. Lehmann D, McFarland R. Overview of Approaches to Mitochondrial Disease Therapy. Journal of Inborn Errors of Metabolism & Screening. 2018;(6):1–7. DOI: 10.1177/2326409817752960.
19. Carlos T. Moraes,etal 1989. Mitochondrial DNA Deletions in Progressive External Ophthalmoplegia and Kearns-Sayre Syndrome. N Engl J Med; 320:1293-1299 DOI: 10.1056/NEJM198905183202001.
20. Lin DD, Crawford TO, Barker PB. Proton MR spectroscopy in the diagnostic evaluation of suspected mitochondrial disease. American Journal of Neuroradiology. 2003 Jan 1;24(1):33-41.
21. Reda HM, Copen WA, Karaa A, Oakley DH. Case 13-2017: A 41-Year-Old Man with Hearing Loss, Seizures, Weakness, and Cognitive Decline. New England Journal of Medicine. 2017 Apr 27;376(17):1668-1678. DOI: 10.1056/NEJMcpc1616022
22. Mancuso M, Orsucci D, Angelini C, Bertini E, Carelli V, Comi GP, Donati A, Minetti C, Moggio M, Mongini T, Servidei S. The m. 3243A> G mitochondrial DNA mutation and related phenotypes. A matter of gender?. Journal of neurology. 2014 Mar;261(3):504-510.
23. Parikh S, Goldstein A, Koenig MK, Scaglia F, Enns GM, Saneto R, Anselm I, Cohen BH, Falk MJ, Greene C, Gropman AL. Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. Genetics in Medicine. 2015 Sep;17(9):689-701.
24. Willems PJ. Genetic causes of hearing loss. New England Journal of Medicine. 2000 Apr 13;342(15):1101-1109. DOI: 10.1056/NEJM200004133421506.

25. Wallace DC. Mitochondrial DNA diseases. Ann Rev Biochem. 1992;61:1175-1212.
26. Zeviani M, Gellera C, Antozzi C, Rimoldi M, Morandi L, Tiranti V, DiDonato S, Villani F. Maternally inherited myopathy and cardiomyopathy: association with mutation in mitochondrial DNA tRNALeu (UUR). The Lancet. 1991 Jul 20;338(8760): 143-147.
27. Johns DR. Mitochondrial DNA and disease. New England journal of medicine. 1995 Sep 7;333(10):638-644. DOI: 10.1056/NEJM199509073331007
28. Nakagawa E, Hirano S, Yamanouchi H, Goto YI, Nonaka I, Takashima S. Progressive brainstem and white matter lesions in Kearns—Sayre syndrome: a case report. Brain and Development. 1994 Sep 1;16(5):416-418.
29. Barragan-Campos HM, Vallée JN, Lo D, *et al*. Brain Magnetic Resonance imaging findings in patients with mitochondrial cytopathies. Arch Neurol. 2005;62:737-742
30. Khambatta S, Nguyen DL, Beckman TJ, Wittich CM. Kearns–Sayre syndrome: a case series of 35 adults and children. International journal of general medicine. 2014;7:325-332.
31. Chinnery PF, DiMauro S, Shanske S, Schon EA, Zeviani M, Mariotti C, Carrara F, Lombes A, Laforet P, Ogier H, Jaksch M. Risk of developing a mitochondrial DNA deletion disorder. The Lancet. 2004 Aug 14;364(9434):592-596. DOI:http://dx.doi.org/10.1016/S0140-6736(04)16851-7
32. Obara-Moszynska M, Maceluch J, Bobkowski W, Baszko A, Jaremba O, Krawczynski MR, Niedziela M. A novel mitochondrial DNA deletion in a patient with Kearns-Sayre syndrome: a late-onset of the fatal cardiac conduction deficit and cardiomyopathy accompanying long-term rGH treatment. BMC pediatrics. 2013 Dec;13(1):27. https://doi.org/10.1186/1471-2431-13-27
33. M. Laloi – Michelin, *et al.* Kearns Sayre Syndrome : an unusual form of Mitochondrial Diabetes. Diabetes and Metabolism. 2006;32(2):182-186. Doi : DM-04-2006-32-2-1262-3636-101019-200517734
34. De Block CE, De Leeuw IH, Maassen JA, Ballaux D, Martin JJ. A novel 7301-bp deletion in mitochondrial DNA in a patient with Kearns-Sayre syndrome, diabetes mellitus, and primary amenorrhoea. Exp Clin Endocrinol Diabetes. 2004;112: 80-83
35. Cat Nguyen Burkat, Michael Clamp 2017. Article Kearns-Sayre Ptosis. EyeWiki American Academy of Ophthalmology,
36. McFarland R, Taylor RW, Turnbull DM. A neurological perspective on mitochondrial disease. Lancet Neurol. Aug 2010;9(8):829-840.
37. Al-Adsani A, Gader FA. Combined occurrence of diabetes mellitus and retinitis pigmentosa. Annals of Saudi Medicine. 2017;10:58.
38. Choi C, Sunwoo IN, Kim HS, Kim DI. Transient improvement of pyruvate metabolism after coenzyme Q therapy in Kearns-Sayre syndrome: MRS study. Yonsei Med J. 2000;41:676-679
39. Remes AM, *et al*. Mitochondrial DNA deletion in dilated cardiomyopathy. A clinical study employing endomyocardial biopsy. JACC. 1994;23(4):93 5-42
40. Schaefer A M, *et al*. Mitochondrial DNA deletion in "identical" twin brothers. Journal of Medical genetics BMJ journals. 2003;41(2):19 http://dx.doi.org/10.1136/jmg.2003.011296
41. Yu N, Zhang Y, Zhang K, Xie Y, Lin X, Qing Di. MELAS and Kearns–Sayre overlap syndrome due to the mtDNA m. A3243G mutation and large-scale mtDNA deletions. eNeurological Sci 2016;4:15–18.
42. Emmanuele V, Silvers DS, Sotiriou E, Tanji K, DiMauro S, Hirano M. MERRF and Kearns-Sayre overlap syndrome due to the mtDNA m.3291T>C mutation. Muscle & nerve. 2011;44(3):448-451. doi:10.1002/mus.22149.
43. Lee-Jun C. Wong, Recognition of mitochondrial DNA deletion syndrome with non-neuromuscular multisystemic manifestation 2001;3(6).
44. Nishigaki Y[1], Tadesse S, Bonilla E, Shungu D, Hersh S, Keats BJ, Berlin CI, Goldberg MF, Vockley J, DiMauro S, Hirano M. A novel mitochondrial tRNA(Leu(UUR)) mutation in a patient with features of MERRF and Kearns-Sayre syndrome, Neuromuscul Disord. 2003 May;13(4):334-340.
45. Shanske S, Moraes CT, Lombes A, *et al*. Widespread tissue distribution of mitochondrial DNS deletions in Kearns-Sayre syndrome. Neurology 1990;40:24-28.
46. Moraes CT, DiMauro S, Zeviani M, *et al*. Mitochondrial DNA deletions in progressive external ophthalmoplegia and Kearns-Sayre syndrome. N Engl J Med 1989;320:1293-1299.
47. Khan NA, Govindaraj P, Meena AK, Thangaraj K. Mitochondrial disorders: Challenges in diagnosis & treatment. *The Indian Journal of Medical Research*. 2015;141(1):13-26.
48. Kosmorsky G, John DR. Neuro-ophthalmologic manifestation of mitochondrial disorder. Neuro Clin 1991;9(1):147-161.
49. DiMauro S, Bonilla E, Lombes A, *et al*. Mitochondrial encephalomyopathies. Neurologic Clinic 1990;6:485-506.
50. Zeviani M, Gellera C, Pannacci M, *et al*. Tissue distribution and transmission of mitochondrial DNA deletions in mitochondrial myopathies. Ann Neurol 1990;28:94-97.
51. Ponzetto C, Bresolin N, Bordoni A, *et al*. Kearns-Sayre Syndrome: Different amounts of deleted mitochondrial DNA are present in several autoptic tissues. J of Neurol Sci 1990;96:207-210.