RESEARCH ARTICLE

DNA Sequence Analysis of High Variable Region HV2b in Kearns-Sayre Syndrome

Mona N. Al-Terehi[1], Mohammed A. Jawad[2], Abed J. Kadhim[3], Monem M. Alshok[4], Ali H. Al-Saadi[5]

[1,5]*College of Science, Babylon University, Hillah, Iraq*
[2,3]*Al-Nisour University College, Baghdad, Iraq*
[4]*College of Medicine, Babylon University, Hillah, Iraq*

Received: 23rd July, 2021; Revised: 03rd August, 2021; Accepted: 11th September, 2021; Available Online: 25th September, 2021

ABSTRACT

Kearns–Sayre syndrome (KSS) is a genetic disorder disease caused by mitochondrial myopathy, and it is a rare syndrome recorded for the first time in Iraq. The present study was carried out to analyze High variable region HV 2b in KSS. A case report study was conducted, a female 19 years old with clinical manifestation of KSS was diagnosed in Margan hospital. Blood was collected from the case and her mother for DNA extraction and mt DNA amplification and sequencing. The results of the present study show that 276 bp of HV 2b, which was sequenced to detect genetic variations and point mutation, also identities with Iraqi placebos and NCBI sequences. The mtDNA sequence analysis shows a single insertion mutation in the KSS case and 11 substitution mutations. The frequency of substitution mutation was disappeared in 11 loci for 7 types of mutation. 22.23% the percentage mutation in 9 loci for 7 types of mutation, the higher percentage was 33.34% of 5 loci for 3 types of mutation. Low-frequency percentage of A, C, GC, AG, AC, TA, CG, CT and (G, C)%, other patterns percentages ware increased T, GG, TG, TT, and (A, T) %. Also, there were no ambiguous nucleotides in the present case and study subjects. The identities between study subjects were in figure 2. The percentage of DNA sequences identities between KSS with NCBI sequence was 87%, KSS with Iraqi placebo 85.77%, KSS and her mother was 79%, case mother with NCBI sequence 98%, case mother with Iraqi placebos 87.55%. the KSS case show branched haplogroups. The present study concluded that there was variation in DNA sequences in KSS case.

Keywords: Haplogroups, Insertion mutation, Kearns-Sayre syndrome, mtDNA, NCBI sequence.

International Journal of Pharmaceutical Quality Assurance (2021); DOI: 10.25258/ijpqa.12.3.18

How to cite this article: Al-Terehi MN, Jawad MA, Kadhim AJ, Alshok MM, Al-Saadi AH. DNA Sequence Analysis of High Variable Region HV2b in Kearns–Sayre Syndrome. International Journal of Pharmaceutical Quality Assurance. 2021;12(3):253-257.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Kearns-Sayre syndrome is a rare disease characterized by dysfunction in multi organs which happened before twenty years,[1] it caused by defects in mitochondria would impaired in oxidative phosphorylation which responsible on cellular energy production.[2] The deletion in mitochondria may be variance between cells, tissue and organs that affected on dysfunction phenotypes this called heteroplasmic pattern.[3] The mitochondrial disorder in KSS syndrome characterized by pigmentary retinopathy, progressive external ophthalmoplegia, also abnormality in muscles, defect in cardiac conditions and endocrinopathies.[4]

The mitochondrial DNA disorder led to defect in protein required for the respiratory chain reaction, some genetic disorders accord in mtDNA like point mutation and deletion a study found that disclosed a 7663-base pair heteroplasmic deletion in the mtDNA 6340–14003 in KSS using Long-range PCR analysis.[5] Studies investigated the inheritance of KSS syndrome, its reported that the inheritance of mitochondrial genome is maternal because during fertilization sperm missed mitochondria with tail.[6]

KSS is rare syndrome it recorded in 1.6 for every 100,000 individuals[7] first described by Kearns and Sayre in 1959,[8,9] in a case report, which presented with external ophthalmoplegia, pigmentary retinopathy and cardiac conduction disorder, in Iraq there was no information's about this syndrome except the present case that reported in Hilla city, Iraq.

MATERIALS AND METHODS

Genetic Study

- *Sample Collection:* about five mL of whole blood was collected from KSS case that admitted to Marjan hospital, the clinical diagnosis's performed by prof. Dr. Monem Makky and case report by Dr. Zahraa Al-Terehi, all data were collected

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

according to ethical approval of ministry of environment and health in Iraq and permission of case, control was collected from healthy contributors, had age (15–22) years, DNA extraction performed using Favor gene extraction kit. Concentration and purity were detected using nanodrope.

- The HV2b of mtDNA was amplification using the following primers (Table 1)
- PCR conditions and size products, PCR experiments performed as a following; per-denaturation for 5 minutes at 94°C, then 35 cycles (30 seconds at 94°C, 30 seconds at 58.4°C, 30 seconds at 72°C, and finally 10 minutes at 72°C). PCR products were determined by electrophoresis pattern in agarose gel (1.5% agarose, 70 V, 20 mA for 45 minutes) with ethidium bromide staining. The PCR size product was 276 for HV2b (192-429) in *Homo sapiens* mitochondrion. Data analysis using 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

Case Report

An 18 years old female presented with a short stature that began when she was 13 with progressive ptosis and bilateral sensorineural hearing loss and Exertional dyspnea, muscle weakness, and wasting that cause loss of 5 kg of her body weight. In reviewing her past hospital admission, she was admitted several months ago due to trendiness and severe headaches. Bp 60/50 mm Hg, CT scan was done at that time. The result was hyper densearea throughout the cerebrum (diagnosed as intracranial calcification); on the physical exam, there is mild hyperpigmentation of the elbow and hirsutism in the face and limbs muscle wasting, and hypotension enlarged thyroid. Pt was conscious, alert, oriented to time 6CUS exam was normal apart from hypotonic ophthalmological exam shows retinal dystrophy with ↓Visual acuity. Serum electrolyte was normal; thyroid function test was ↓T4 level with normal TSH and ↑CK. Liver function test was normal and normal urine appearance under microscopic, ESG show 2:1 antrioventricular (AV) block with intermittent complete AV block left bundle branch block (LBBB). Family history, her father HTN, recumbent pulmency inf+ allergy, DM.

Table 1: Primers sequences used in mtDNA amplification sites.

HV2b C2 (L 177)	F- TTA TTT ATC GCA CCT ACG TTC AAT
D1 (H 409)	R- CTG TTA AAA GTG CAT ACC GCC

Genetic Test

DNA Extraction, PCR Products, and Virtual Amplification

DNA extraction and PCR products were shown in Figure 1, amplification products of HV 2b were 276 bp for study subjects. This deal with virtual amplification performed by bioinformatics software which shows in Table 2.

Multiple Comparisons

The DNA sequence analysis of the case show variant results, as shown in tables and figures. The multiple Comparisons of mitochondrial DNA HV2b between case and NCBI sequence, case mother and Iraqi placebos show variant polymorphisms as shown in Figure 2 and Table 3. There was a single insertion mutation in KSS case compare with NCBI sequence while it corresponding with Iraqi placebos at loci 253, eleven substitution mutations in KSS case; ten of them were corresponding with NCBI and Iraqi placebos while one of them was differenced, in Iraqi placebos was G while in NCBI was A, and it was T in KSS case. The frequency of substitution mutations was ranged (0–33.34)%. It was diapered in 11 loci for 7 types of mutations. 22.23% was the percentage

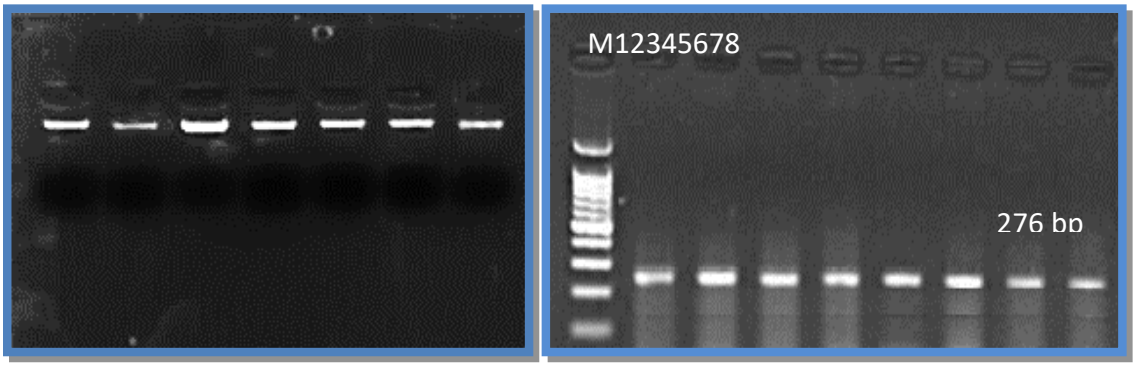

Figure 1: The electrophoresis pattern of DNA extraction and PCR product of HV2b amplification (276) bp for study subjects,1% agarose,75V,20Am for 1-hour. Lane M DNA marker, lane 1-6PCR product OF Iraqi placebos, lane 7,8 PCRproducts of KSS case and her mother.

Table 2: DNA sequences of virtual amplification of HV 2b with PCR size products.

Loci	*DNA sequenceof virtual amplification*	*Size products*
HV2b	TTATTTATCGCACCTACGTTCAATATTACAGGCGAACATACTTACTAAAGTGTGTTAATTAATTAATGCTT GTAGGACATAATAATAACAATTGAATGTCTGCACAGCCACTTTCCACACAGACATCATAACAAAAAATTT CCACCAAACCCCCCCTCCCCCGCTTCTGGCCACAGCACTTAAACACATCTCTGCCAAACCCCAAAAACA AAGAACCCTAACACCGCCTAACCAGATTTCAAATTTTATCTTTTGGCGGTATGCACTTTTAACAG	276

of appeared mutations in 9 loci for 7 types of mutations, the higher percentage was 33.34% it has appeared in 5 loci for 3 types of mutation.

The DNA State

Table 4 clarified the DNA state of KSS, NCBI, Iraqi placebos, and case mother, low percentages of some patterns could be seen in the frequency of A, C, GC, AG, AC, TA, CG, CT, and (G, C)%. In other patterns, percentages ware increased like the frequency of T, GG, TG, TT and (A,T)%, on the other hand, other patterns were varied when compared with study subjects; also, there were no ambiguous nucleotides in the present case and in study subjects.

DNA Identities

The identities between study subjects were explained in figure 3. The percentage of DNA sequences identities between KSS with NCBI sequence was 87%, KSS with Iraqi placebos 85.77%, KSS and her mother was 79%, case mother with NCBI sequence 98%, case mother with Iraqi placebos 87.55%, Iraqi placebos with NCBI DNA sequence 96.22%.

Phylogenic Tree

The phylogenic tee of study subjects show in figure (3), there were two haplogroups groups it were (10) and other study subjects in other haplogroups branches, one of these was KSS case while other branches consist of two sub branches including KSS case mother and seven of Iraqi placebos.

DISCUSSION

The present study aimed to investigate the variation in DNA sequence of KSS case in the Iraqi population; this case is the first patient recorded in Iraq according to the Iraqi ministry of environment and health. Thus the present study was suggested, the previous studies improvement that this syndrome was rare, Yu *et al*. recorded one patient (male) in Chins,[10] in brazil there were two twins sister of KSS.[11] There is no information's about this syndrome in Arab countries.

The variations in mtDNA sequences of KSS case shown in the Table 3 and 4 and Figure 2, these variations in the case may be responsible for some case phenotypes which studies have improved. Although observation of mutated mtDNA in lymphocyte of blood does not associate with the severity of clinical symptoms of the KSS[8], investigators explained that mutation or deletion in mtDNA was dissimilar between tissues and organs which known heteroplasmic.[12]

One of the studies show deletion in mtDNA approximately 6.7 kb in two patients of KSS using southern blot and PCR[13] in the present study, and there was no deletion in KSS case this because the loci which was analyzed in the present study had substitution mutation in addition to one insertion mutation. Thus, the case needs more investigation about other loci's of mtDNA. In the other hand, HV1a and HV2a were analyzed for the same case (data not shown); the results of these loci's show variation in sequences, and no large-scale deletion was observed. However, the low percentage of the mtDNA was deleted because of the low level of mutated mtDNA in blood and sometimes cannot be detected.[14] Di Mauro *et al*. clarified that the deletions in mtDNA occurred de novo in the mother's oocytes or during embryogenesis.[15]

In the present study KSS show, low identity with her mother mtDNA sequences 79%, while she showed it approximated identities with NCBI and Iraqi placebos (87%, 85.77%). Case mother show low identity with Iraqi placebos also, from these percentage can be concluded that KSS was inheritance some mutation from her mother while other mutations were induced in case during an embryonic period or childhood. The environmental in Iraq supported induced mutation in DNA because of increment in pollution in air, soil, and water, in addition of stress and low power of lifestyle for persons A big studies in Iraq show that there were de nova mutations in genes led to incidence diseases like cancer, auto immune disease, in addition of hypertension and progressive of diabetic complication, also incredible of oxidative stress.[16-20] Also, the data recorded by Khambatta *et al*. that none of the 35 patients with KSS had a family history of KSS.[21]

The phylogenic tree shows the differences of KSS case with other study subjects, although of her contributed in the same

Table 3: Variations in HV1a of Kearns-Sayre syndrome

Loci variant	*NCBI >KSS*	*Iraqi placebos>KSS*	*Frequency in Iraqi placebos (%)*
204, 205	A>G	A>G	0, 22.23
207, 210, 224	G>T	G>T	0, 11.12, 0
209, 421	T>G	T>G	11.12, 33.34
225, 236,242	C>G	C>G	11.12, 0,0
230, 274, 342	A>T	A>T	0, 22.2, 0
235, 276, 280	A>C	A>C	0, 22.3, 22.3
253	Insertion T	-	0
257, 418	C>G	C>G	0, 33.34
262, 289, 376	C>A	C>A	22.2, 22.23, 22.23
265, 268, 299, 316, 352	C>T	C>T	0, 22.3, 33.34, 33.34, 11.12
276	A>C	A>C	22.23
279	G>A	G>A	22.23

Table 4: The DNA sequence stats of study subjects

Pattern:	*NCBI sequence (%)*	*Iraqi placebo (%)*	*Case (%)*	*Case mother (%)*
g	11.27	14.145	13.67	12.50
a	34.18	33.54	30.08	33.98
t	26.55	25.10	30.86	24.61
c	28.00	27.20	25.39	28.91
gg	1.82	3.945	5.10	1.96
ga	2.19	3.4	2.35	3.14
gt	2.55	3.17	3.53	2.75
gc	4.38	4.33	2.75	4.71
ag	3.28	3.89	2.35	4.31
aa	13.14	14.66	14.12	13.33
at	7.66	6.39	6.27	6.67
ac	10.22	8.31	7.06	9.80
tg	4.01	4.25	5.49	4.71
ta	8.03	6.44	4.71	7.06
tt	10.22	9.34	16.47	9.02
tc	4.38	3.85	4.31	3.92
cg	2.19	1.52	0.78	1.57
ca	10.95	8.85	9.02	10.20
ct	5.84	5.682	4.31	6.27
cc	9.12	11.251	11.37	10.59
g,c	39.27	41.35	39.06	41.41
a,t	60.73	45.68	60.94	58.59
r,y,s,w,k*	0.00	0	0.00	0.00
b,h,d,v,n*	0.00	0	0.00	0.00
r,y,s,w,k,m,b,d,h,v,n*	0.00	0	0.00	0.00

Ambiguous nucleotides

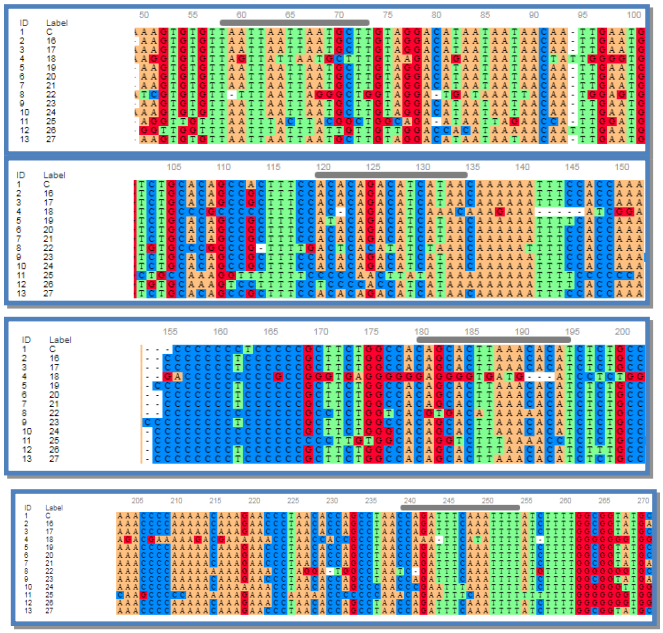

Figure 2: Multiple comparisonsof mitochondrial DNAHV2b, lane 2-11 Iraqi place bos sequences 12, case 13 case mother, C NCBI sequence ID: NC_012920.1.

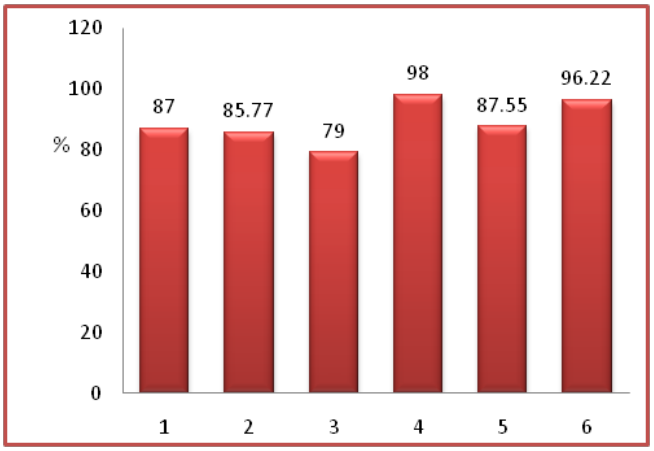

Figure 3A: The percentage of DNA sequences identities between study subjects lane 1 KSS with NCBI sequence; lane 2 KSS with Iraqi placebo; lane 3 KSS and her mother; lane 4 case mother with NCBI sequence; lane 5 case mother with Iraqi placebo; lane 6 Iraqi placebo with NCBI DNA sequence.

root, it had branched haplogrupe from other study subjects because of point mutations that recorded in the present study also differentiations in DNA states made this haplogrupe.

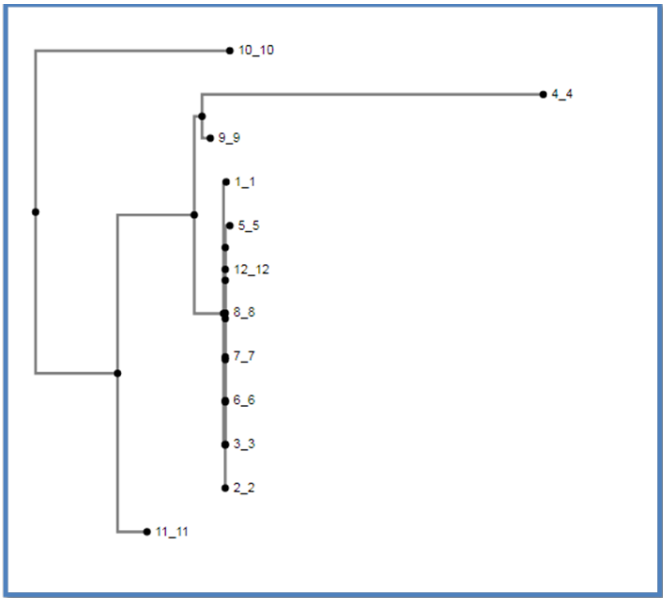

Figure 3B: Phylogenic tree of HV2bin mtDNA of study subjects, 1 NCBI sequence, 2-10 Iraqi placebos, 11 KSS case, 12 case mother.

Some point mutations were recorded, such as m.3243A>G [22] or m.3249G>A.[23]

REFERENCES

1. Maceluch JA, Niedziela M: The clinical diagnosis and molecular genetics of Kearns-Sayre syndrome: a complex mitochondrial encephalomyopathy. Pediatr Endocrinol Rev. 2007; 4:117–137
2. Kearns-Sayre syndrome. *Genetics Home Reference (GHR)*. December 2011; http://ghr.nlm.nih.gov/condition/kearns-sayre-syndrome.
3. Helen AL. Tuppen, Emma L. Blakely, Douglass M. Turnbull, Robert W. Taylor Mitochondrial DNA mutations and human disease, Biochimica et Biophysica Acta. 2010; 1797 , 113–128.
4. Artuch R, Pavía C, Playán A, Vilaseca M A, Colomer J, Valls C, Rissech M, González M, A, Pou A, Briones P, Montoya J, Pineda M, Multiple Endocrine Involvement in Two Pediatric Patients with Kearns-Sayre Syndrome. Horm Res 1998;50:99-104.
5. 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, 13:27
6. Laloi-Michelin M., Virally M., Jardel C., Meas T., Ingster-Moati I., Lombes A., Massin P. Guillausseau PJ. Kearns Sayre syndrome: An unusual form of mitochondrial diabetes. Diabetes and Metabolism, 2006; 32 (2) , 182-186.
7. Nasseh IE, Tengan CH, Kiyomoto BH, Gabbai AA. Doenças mitocondriais. Rev Neurociências. 2001;9(2):60-9.
8. Kearns TP, Sayre GP. Retinitis pigmentosa, external ophthalmophegia, and complete heart block: unusual syndrome with histologic study in one of two cases. AMA Arch Ophthalmol. 1958; 60(2):280-9.
9. Timothy J. Young Amitk. Shah Mun Heng Lee David L. Hayes. Kearns-Sayre Syndrome: A Case Report and Review of Cardiovascular Complications. 2005;28(5):454-457
10. Yu, N. Yan-fang Zhang, Kang Zhang, Yuan Xie, Xing-jian Lin, 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.
11. Oliveira L, Karina CB, Eduardo BO, Kátia FA. Cochlear implant in Kearns-Sayre syndrome: case study of twin sisters. Audiol Commun Res. 2017;22:e1841
12. Mohri I, Taniike M, Fujimura H, Matsuoka T, Inui K, Nagai T, Okada S. A case of Kearns-Sayre syndrome showing a constant proportion of deleted mitochondrial DNA in blood cells during 6 years of follow-up. J Neurol Sci. 1998 Jun 11;158(1):106-9.
13. Kokotas H, Petersen MB, Willems PJ. Mitochondrial deafness. Clin Genet. 2007;71(5):379-91.
14. Holt IJ, Harding AE, Morgan-Hughes JA: Deletions of mitochondrial DNA in patients with mitochondrial myopathies. Nature 1988, 331:717–719.
15. Di Mauro S, Hirano M. Mitochondrial DNA Deletion Syndromes. [Updated 2011 May 3]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. Gene Reviews®. 2003; 17
16. Al-Terehi M, Hasan AH, Muhammed HJ, Mohsen IH, Al-Saadi AH, Zaidan HK, Al-Kiam ZH. Polymorphisms of Glutathione-S-Transferase M1 and T1 Genes in Breast Cancer Tissue in Iraqi patients. JCPS. 2016;9(4):2911-2914.
17. Al-Terehi M, Hasan A, AL-Jboory M, Al-Saadi 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
18. Al-Terehi M, Ghaleb R, Al-Oubaidy SH, Al-Saadi A, Zaidan H. Study *TNF-α* gene polymorphism in Type 1 Diabetic Patients Using ARMS technique Journal of Chemical and Pharmaceutical Science CPS. 2016; 9,3,1107-1111.
19. Al-Terehi M, Al-Saadi A, Zaidan H, Al-shariafe A. Theoretically Construction Expression Vector Consist of Two Reporter Gene as a Fusion Protein, Australian Journal of Basic and Applied Sciences. 2015;9(7):586-595.
20. Obara-Moszynska M, Maceluch J, Bobkowski W, et al. 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 Pediatr*. 2013;13:27. Published 2013 Feb 20.
21. Khambatta S, Nguyen DL, Beckman TJ, Wittich CM. Kearns-Sayre syndrome: a case series of 35 adults and children. Int J Gen Med. 2014; 7: 325-332.
22. Wilichowski E, Korenke GC, Ruitenbeek W, De Meirleir L, Hagendorff A, Janssen AJ, et al. Pyruvate dehydrogenase complex deficiency and altered respiratory chain function in a patient with Kearns-Sayre/MELAS overlap syndrome and A3243G mtDNA mutation. J Neurol Sci 1998;157:206-213.
23. Seneca S, Verhelst H, De Meirleir L, Meire F, Ceuterick-De Groote C, Lissens W, et al. A new mitochondrial point mutation in the transfer RNA(Leu) gene in a patient with a clinical phenotype resembling Kearns-Sayre syndrome. Arch Neurol 2001;58:1113-1118.