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Original Research Article

Association of Fibroblast Growth Factor (Fgf-21) as a Screening Biomarker for Chronic Progressive External Ophthalmoplesia

Farnaz Farzaneh Dehkordi[1*], Massoud Houshmand[2], Majid Sadeghizadeh[3], Mohsen Bahmani Kashkouli[4] and Gholamreza Javadi[1]

[1]Biology Department, Science and Research Branch, Islamic Azad University, [2]Medical Genetics Department, National Institute of Genetic Engineering and Biotechnology, [3]Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, [4]Iran University of Medical Sciences, Tehran, Iran.

For correspondence: **Email: farzaneh_farnaz2007@yahoo.com; **Tel:** +98-9127336690*

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Abstract

Purpose: *To investigate whether or not fibroblast growth factor (FGF-21) can be used as a screening biomarker in chronic progressive external ophthalmoplesia (CPEO) patients.*
Methods*: FGF-21 concentration was measured in the serum of 24 patients with CEPO phenotype and 24 control samples by enzyme-linked immunosorbent assay (ELISA) and determined the deletion of mitochondrial genome by multiplex polymerase chain reaction (PCR).*
Results: *FGF-21 concentration in 50 % of CPEO patients showed notable differences from that in control subjects. FGF-21 concentration ratio in patient group, 2 disorder control groups (mitochondrial and non-mitochondrial) and normal group, respectively, was 294.87 ± 42.10 ($p < 0.0001$), 761.78± 75.07 ($p < 0.0001$), 124.26 ± 12.27 ($p = 0.1203$), 69.27 ± 10.09 ($p = 0.2195$). A statistically significant inverse correlation between FGF-21 concentration and age onset was found, with a significant difference ($p < 0.05$) in the age group ≤ 19 years (mean FGF-21 concentration, 460.36 pg/mL) and for the age group ≥ 51years (mean concentration FGF-21, 57.87 pg/mL. Surprisingly, there was no significant difference between FGF-21 concentration and age in the mid-age group (20 – 50 years) .*
Conclusion: *These findings indicate that FGF-21 concentration significantly increases in CPEO patients like in other mitochondrial disorders and this factor can be used as a biomarker in primary diagnosis of mitochondrial disorders. In this regard, FGF-21 assay is only valid in teenagers and the > 50 years age group who show acute symptoms.*

Keywords: *Chronic progressive external ophthalmoplesia, Fibroblast growth factor-21, Mitochondrial disorders, Ophthalmoplesia, Biomarker.*

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INTRODUCTION

Mitochondrial diseases include a group of metabolic disorders that affect people of all ages [1]. Based on subsarcolemmal accumulations of abnormal mitochondria and their intense red appearance with histological staining, mitochondrial diseases are divided in two major groups; ragged-red fiber disorders and non-ragged-red fibers ones. The first group includes diseases such as Kearns-Sayre syndrome (KSS), mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged red fibers (MERRF), Pearson syndrome, progressive external ophthalmoplegia (PEO) [2,3].

CPEO is a mitochondrial myopathy that causes muscular or multisystem symptoms [4]. The disease is characterized by a progressive involvement of the ocular musculature, with ptosis of the eyelids and impairment of eye movements [4,5]. Lactate, pyruvate, aminoacids and creatine kinase are considered as serum markers for the diagnosis of mitochondrial disorders. However, since they do not have a good sensitivity and specificity for such a detection muscle biopsy is considered as a better tool for detecting extensive intra-muscular mitochondrial damages [6-8]. On the other hand, biopsy is an invasive procedure that is more costly than testing a serum biomarker; furthermore, children would require a general anesthesia in biopsy.

Suomalainen *et al* introduced fibroblast growth factor 21 (FGF-21) as a new serum biomarker in patients with mitochondrial dysfunctions [7]. Consequently, the aim of this study was to determine if the measurement of FGF-21 concentration in the serum of Iranian patients with CPEO can be used as a biomarker for human mitochondrial disorder.

EXPERIMENTAL

Patients

24 patients (14 men and 10 women) with CPEO were referred to the Genetics Department at Special Medical Centre (SMC) of Tehran for diagnosis. Documented, written consent was obtained from all patients who agreed to participate. The study protocol was approved by SMC project Nr 385 ethics committee according to World Medical Association's Declaration of Helsinki [9]. Our study was performed according to WHO criteria [10]. Serum samples were collected from CPEO patients and control groups, comprised of two groups of non-mitochondrial (control group 1) and other mitochondrial disorders except CPEO (control group 2), e.g., mitochondrial myopathy, Leber's hereditary optic neuropathy (LHON), mitochondrial encephalomyopathy, lactic acidosis, and stroke (MELAS); control group 2 consisted of 9 mitochondrial and 5 non-mitochondrial disorder patients,while the healthy control group comprised of 5 men and 5 women. These cases are listed in Table 1 and Table 2.

FGF-21 concentration measurement

ELISA (BioVendor Human FGF-21 ELISA Kit) was used to measure FGF-21 concentration in serum samples. For FGF-21, the cutoff was based on the reference ranges stated in the study of the Suomalainen *et al*. [7]. Thus, the cutoff value used ws 200 pg/mL.

Molecular study

Due to the fact that in most cases, CPEO occurs as result of a sporadic deletion of mitochondrial genome, multiplex polymerase chain reaction (PCR) was used to study mtDNA in patients who showed symptoms of the disease. Six primers were used to analyze the whole mitochondrial genome.

A pair of primers related to the mtDNA region that is generally not deleted was used as internal control, and the others were used to show delete regions. The PCR of each sample was set in a 0.2 ml tube using 100 mg of total DNA, 10 pmol of each primer, 200 mmol of dNTPs, 1X PCR buffer containing 2.5 mmol MgCl2 and 1 U Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany). The cycling conditions used for the amplification were as follows: initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 55 °C and extension for 35 sec at 72 °C (final extension for 10 min).

Statistical analysis

$P < 0.05$ was considered statistically significant and this was assessed using Duncan's multiple range test to compare FGF-21 concentrations between patients and controls. Also compared were FGF-21 concentrations between the 3 age groups (≤ 19 years 20 - 50 years and ≥ 51 years) in CPEO patients. Data are expressed as mean ± standard error (SE).

RESULTS

The concentration of FGF-21 in 50 % of cases (total: 48) with CPEO phenotype was not in the normal range. While there was no increase in FGF-21 concentration in healthy and non-mitochondrial disorder control groups, a higher concentration was detected in the mitochondrial disorder groups (CPEO and other mitochondrial disorders). The outcomes of statistical comparisons of the pertinent parameters are shown in Tables 3 and 4.

Genetic analysis showed that 50 % of patients with CPEO clinical symptoms as well as increased serum FGF-21 concentration, there was deletion of mtDNA, while in the other patients with CPEO clinical symptoms and normal serum FGF-21 concentration, there was no deletion of mtDNA. The observed bands on

Table 2: Serum FGF-21 concentrations in 3 control groups; disease control group (non-mitochondrial: mental retardation, epilepsy, dismorphia, DMD and other mitochondrial disorders: LHON, MELAS, mitochondrial myopathy) and healthy control group.

Case no.	Sex	Age (years)	Clinical feature	FGF-21(pg/ml)
1	F	44	LHON	789.8
2	F	52	LHON	1549
3	M	17	LHON	451.6
4	M	22	LHON	824.0
5	F	20	MELAS	723.1
6	M	51	MELAS	612.8
7	M	55	Mitochondrial myopathy	619.0
8	F	27	Mitochondrial myopathy	830.0
9	F	36	Mitochondrial myopathy	456.8
10	F	28	Mental retardation	62.4
11	F	18	Epilepsy	169.1
12	F	22	Epilepsy	151.3
13	M	38	Dismorphia	128.2
14	M	29	DMD	110.3
15	F	15	Healthy	48.2
16	F	43	Healthy	72.7
17	F	23	Healthy	24.4
18	M	45	Healthy	16.7
19	F	56	Healthy	112.5
20	M	47	Healthy	177.0
21	M	52	Healthy	56.7
22	F	32	Healthy	48.3
23	M	26	Healthy	72.9
24	M	34	Healthy	63.3

Table 3: Statistical comparison of FGF-21 factor between 4 groups; (group 1: CPEO patients; group 2: other mitochondrial disorders; group 3: non-mitochondrial disorders; and group 4: healthy control group)

Group	Minimum concentration	Maximum concentration	Mean ± SE	Duncan grouping	*P*-value
1	8.20	849.80	294.87± 42.10	B	<.0001
2	451.60	1549.00	761.78± 75.07	A	<.0001
3	62.40	169.10	124.26±12.27	C	0.1203
4	16.70	177.00	69.27±10.09	C	0.2195

Table 4: Statistical comparison of FGF-21 factor between 3 age groups; (group 1: ≤ 19 years old; group 2: 20 - 50 years old; and group 3: ≥ 51 years old).

Group	Frequency	%	Minimum concentration	Maximum concentration	Mean ± SE	Duncan grouping	*P*-value
1	6	25.00	90.40	849.80	460.36±115.14	A	0.0005
2	14	58.33	8.20	836.40	291.66±81.58	A,B	0.0007
3	4	16.67	42.50	88.80	57.87±10.49	B	0.6802

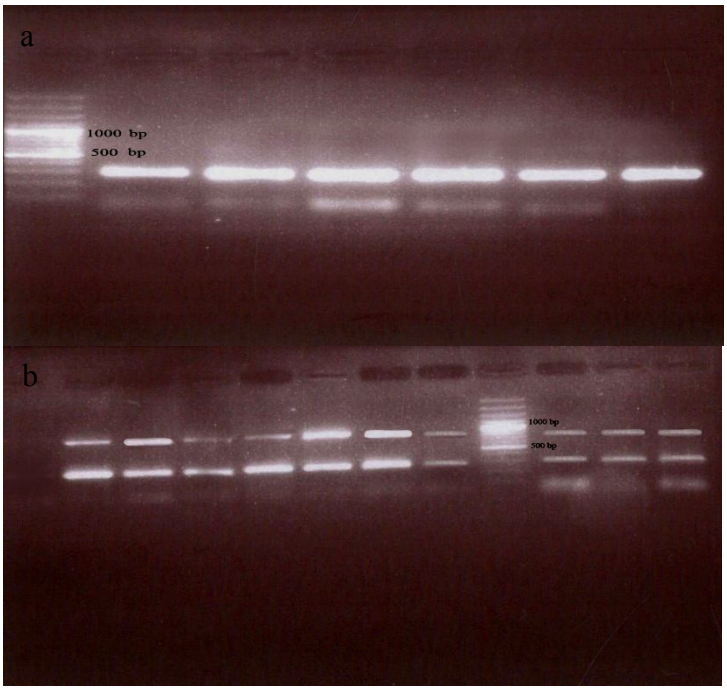

Figure 1: Multiplex PCR using ONP 86, ONP 89 and ONP 10 primers. a = left to right; 5 first are related to CPEO patients without increase in FGF-21 concentration and the other is related to normal individuals. They show only the internal control band in size of 279bp; B = The bands are related to CPEO patients with increase in FGF-21 concentration. They all show 2 bands, 279bp band is related to ONP 86 and ONP 89 primers as a internal control and the upper band is related to ONP 66 and ONP 10 primers that polymerase the DNA when the deletion was occurred. The size of this band is 870 bp.

agarose gel electrophoresis showed the existence of deletion in the region between two primers (Figure 1). The size of the deleted region in the CPEO patients with increase in FGF-21 concentration was 870bp.

DISCUSSION

Mitochondrial disorders are those types of metabolic diseases that lead to decrease of energy in damaged organs due to defect in respiratory chain complexes. However, brain, kidney, liver and muscles are the organs that use most of the body energy and so are more prone to damage [11]. According to the justification of Suomalainen *et.al* [7], FGF-21 is the factor that plays an important role in lipid metabolism and also FGF-21 concentration increases in individuals with mitochondrial disease such as MELAS, mitochondrial recessive ataxia syndrome (MIRAS), mitochondrial neurogastro-intestinal encephalopathy (MNGIE); the authors proposed this factor as a serum biomarker for the diagnosis of mitochondrial disorders [7,12], due to the diffulty in carrying out eyelid muscular biopsy in CPEO patients with ophtalmoplasia phenotype. This is why the concentration of FGF-21 as a biomarker in this type of mitochondrial diseases was undertaken in this study.

The results obtained in this study show that FGF-21 could become a suitable general biomarker for the diagnosis of mitochondrial diseases. These findings reveal that FGF-21 concentration will significantly increase in CPEO patients like other mitochondrial disorders and hence this factor can serve as a biomarker in the primary diagnosis of mitochondrial disorders. We also suggest 200 pg/mL as a cutoff concentration.

Increased concentration of the test factor in 50 % of cases with confirmed CPEO phenotype was observed and that it was not gender-dependent; however, with regard to age groups, significant differences were observed except for the 20 – 50 years age group.

Although genetic studies on the patients who showed increased FGF-21 concentration demonstrated partial deletion of mtDNA, similar deletion in the other 50 % of patients who exhibited normal FGF-21 concentration This finding could suggest another genetic factor other than mtDNA deletion in the phenotype of ophtalmoplasic patients, or misdiagnosis of the patients. It must be mentioned that Suomalainen *et.al*'s study was on patients who definitely suffer from mitochondrial disorders. In the present, we had no access to histological data on the eyelids and definite biochemical markers such as COX1

for diagnosis of mitochondrial disorders in Iran. Nonetheless, this study suggests the possibility of the application of FGF-21 assay for diagnosis of patients who definitely suffer from mitochondrial disorders.

CONCLUSION

It is postulated that FGF-21 factor could serve as a serum biomarker for the early diagnosis of CPEO in patients with definite mtDNA deletion. The low population rate of this phenotype in Iran would limit a definitive statement on the relationship between the genotype of this disease (deletion or non-deletion of mtDNA) and increased FGF-21 concentration. However, our findings indicate that the application of serum FGF-21 concentration not only eliminates the need for muscular biopsy but also can be used as a predictive test for those CPEO patients who have deletion in mtDNA but could not be distinguished from other similar clinical phenotypes (phenocopies).

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