How to Cite:
Sathya, S., Bupesh, G., & Vasanth, S. (2022). Mitochondrial dna copy number by RT-PCR in patients with gestational diabetes mellitus in south Indian population. *International Journal of Health Sciences*, *6*(S7), 2811–2820. https://doi.org/10.53730/ijhs.v6nS7.12127

Mitochondrial dna copy number by RT-PCR in patients with gestational diabetes mellitus in south Indian population

V. Sathya
Research Scholar, Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education and Research, Chromepet, Chennai - 600 044, Tamil Nadu, India

Giridharan Bupesh*
Department of Forest Science, Nagaland University, Zunheboto - 798 627, India

Dr. S. Vasanth*
Research Scientist, R & D Wing, Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education and Research, Chromepet, Chennai - 600 044, Bharath Institute of Higher Education and Research
Tamil Nadu, India
*Corresponding author email: sakthivel.vasanth@gmail.com

***Abstract*---**Mitochondria have its own DNA called Mitochondrial DNA (mtDNA)). There are many reasons behind Mitochondrial dysfunction, may be due to point mutations, deletions, and variations in Mitochondrial DNA copy number. Pregnant women affected with GDM are associated with some alterations in mitochondria DNA copy number (mtDNA-CN). The aim of this study is to check the changes in mtDNA-CN using real-time PCR in pregnant women who are diagnosed with GDM. 25 GDM affected pregnant women and 25 healthy pregnant women were enrolled for this study. Peripheral mitochondrial copy number was checked by Real Time PCR . The result shown a mild decrease in mtDNA-CN in the pregnant women who are affected with GDM when compared to the healthy pregnant women. This reduction or variation in mitochondria DNA CN can leads to mitochondrial dysfunction.

***Keywords*---**Gestational diabetes mellitus, Mitochondrial dysfunction, Mitochondrial DNA copy number, RT-PCR ,Metabolic disorders.

Introduction

Mitochondrial dysfunction contributes to many mitochondrial diseases and a variety of aging-based pathologies as well. Mitochondria have their own genomes (mitochondrial DNA (mtDNA)) and the abnormalities, such as point mutations, mitochondrial copy number variations and deletions, are involved in dysfunction of mitochondrial. Psychosocial stress is known to contribute to oxidative stress in the placenta. Many researches had showed that maternal mtDNA copy number is associated with birth weights of babys.[1] Many studies suggested the hypothesis that mitochondrial DNA copy number may possibly serve as an early marker of health risks especially in pregnant women.1Mitochondrial DNA copy number (mtDNA-CN), is the measure of number of mitochondrial genomes per cell, is a most significant measure for checking the functions of Mitochondria and is connected with many aging-related diseases too.[2]

The mitochondrial copy number per cell is purely based on the energy demand of the cell. For example muscle cells and sperm cells have many mitochondria for performing contractile and motility function. So, the estimated range of mt DNA is from hundreds to one thousand mitochondria per cell, with 2 to 10 DNA copies per mitochondrion.[3] Results from so many studies have shown that smoking, hypercholesterolemia, and obesity can induce mitochondrial DNA damage and dysfunction. [4] Mitochondrial mutations is likely to contribute to a number of common diseases. The common example is diabetes mellitus, which is the most prevalent disease of carbohydrate metabolism affecting human race world wide.[5]

Females are most affected than males due to the maternal pattern of mitochondrial inheritance. It is very important to remember that there are many mitochondria within a cell, will have its own mtDNA and potential mutations too. so, when we are discussing about mitochondrial mutations, it is necessary to think about possibility of mutations present in the entire mitochondrial population rather than in a single mitochondrion.[5,6]

Gestational diabetes mellitus (GDM) is a metabolic disorder which is due to glucose intolerance with the onset or first recognition at the time of pregnancy.Carbohydrate intolerance in pregnant women happens during the excess intake of food to guarantee the proper nutrient supply for the feotus. GDM is due Insufficient insulin to meet the demand so the Normal glucose homeostasis is disturbed. [7,8,9]

Materials and methods:

The Study population includes 25 GDM diagnosed pregnant women and 25 healthy pregnant women without any known complications with an age group between 20 to 36.

Inclusion criteria: Pregnant women who are willing for the study with history of Diabetes for the first time during pregnancy and Pregnant women with obesity.

Exclusion criteria: Patients who are not willing for the study and having H/O Type 1 & Type 2 DM. H/O Existing thyroid disorders, Pregnant women who are

affected with chronic diseases like carcinoma, tuberculosis, kidney diseases and advanced liver failure like cirhosis etc. were excluded from our study.

Mitochondrial DNA -CN was measured by amplifiying ND1 gene in mitochondrial DNA and nuclear gene 18s by Real Time PCR technique.[10,11] Two pairs of primers were designed and used in two steps for quantification of mitochondrial DNA. One primer pair was to amplify MT-ND1 gene in mitochondria and other primer sequence to amplify single-copy nuclear gene 18s.The ND1 to18s CN ratio was determined from standard curve for each sample. [12,13,14] This ratio is proportional to the mtDNA -CN in each cell. The ratio for each sample was then normalized to calibrator DNA in order to standerdize between different runs. The calibrator DNA is a genomic DNA sample from healthy control subjects which is used for comparison of results of different independent assays.[15,16] The DNA sample which is extracted from molecular lab is retrieved and used for the study.[17]

Setting up standards: For each standard curve one reference DNA sample is diluted 1:4 to produce a seven-pont standard curve. measure the initial concentration of reference DNA by nanodrop.[18] A standard curve consisting of diluted reference DNA , a negative control and a calibrator DNA was included in each run. For all standard curve one reference DNA sample was serially diluted to 1:2 to produce a seven point standard curve between 0.3125 and 20 ng of DNA.

controls: 1.2819(calibrator) final concentration – 0.1 ng/µl, 2. 2888 (control)final concentration - 0.1 ng/µl. [19,20]

Setting up 96 well plate for RT-PCR.:

All the samples were assayed in duplicate on a 96 –well plate with an applied Biosystems 7900 sequence detection system. The PCR for mitochondrial DNA and nuclear gene were as usual performed on 96 -well plates with the same samples in the same well positions to avoid position effect. [21,22] A standard curve with diluted reference DNA, one positive control and one calibrator DNA were included in each run. All the test samples are scanned before platting in the same order as they run on plates. Then prepare PCR mixture in a total of 14 µl per well for 96 well plate.[23,24,25]

Results

Table: 1 Log ND1 Standard Concentration

	ND1 standards	log	ave Ct-ND1	stdev ND1		
BT1	15	1.176091	16.81	0.070711	slope	-3.62802
BT2	3.75	0.574031	18.34	0.141421	intercept	20.81831
BT3	0.9375	-0.02803	21.06	0.056569		
BT4	0.234375	-0.63009	23.05	0.240416		
BT5	0.05859375	-1.23215	25.38	0.127279		
BT6	0.014648438	-1.83421	27.32	0.06364		

BT7	0.003662109	-2.43627	29.77	0.077782		
BTBLANK						

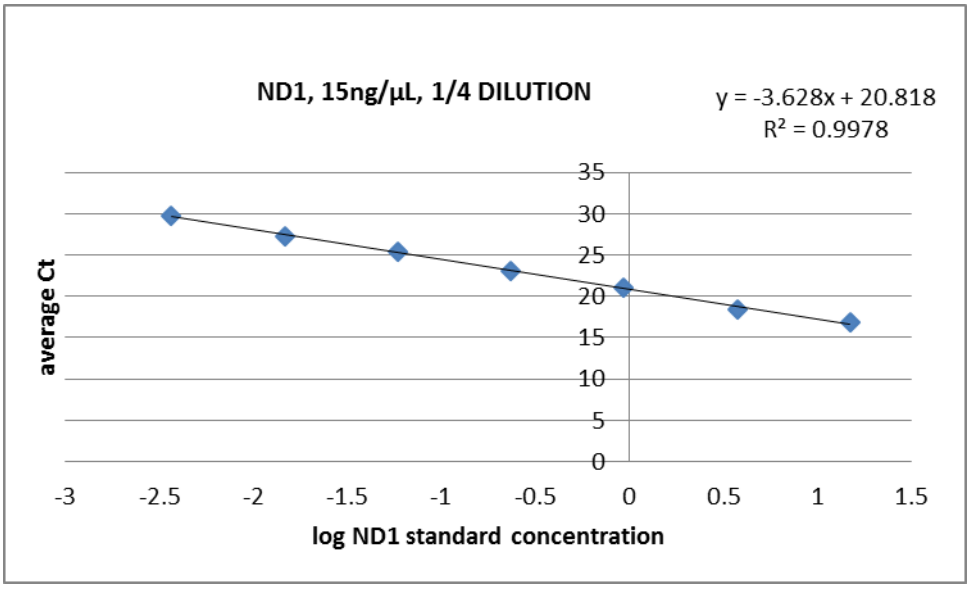
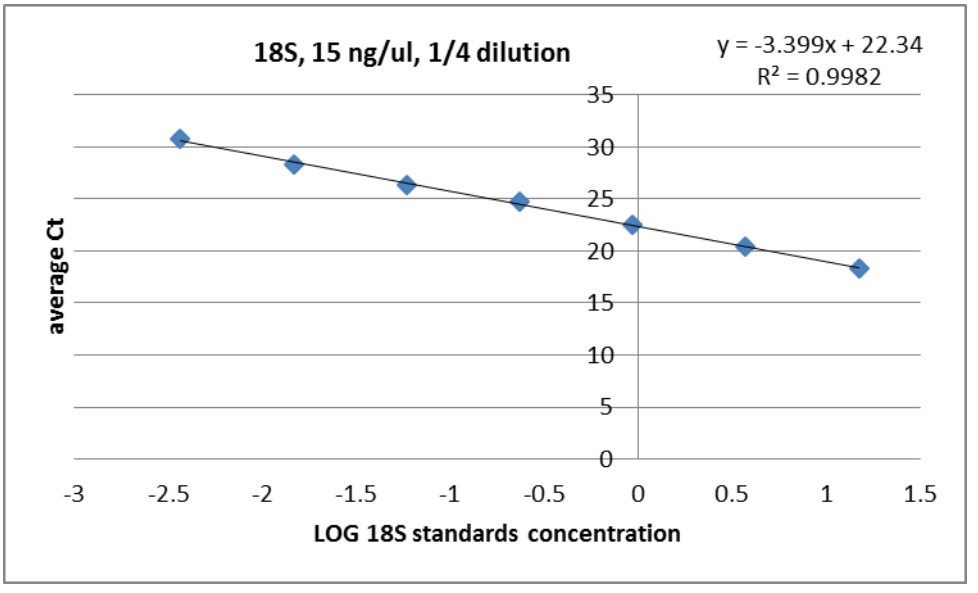


Fig: ND1 Standard Concentration

Table;2 Log 18S Standard Concentration

	18S standards	log	ave Ct-18S	stdev-18s		
BT1	15	1.176091	18.275	0.035355	slope	-3.39904
BT2	3.75	0.574031	20.395	0.06364	intercept	22.34044
BT3	0.9375	-0.02803	22.495	0.338346		
BT4	0.234375	-0.63009	24.72	0.328727		
BT5	0.05859375	-1.23215	26.395	0.103755		
BT6	0.014648438	-1.83421	28.27	0.067466		
BT7	0.003662109	-2.43627	30.825	0.049497		
BTBLANK						



Fig;2 Log 18S Standard Concentration

T11	22.17	0.24	-0.37	0.42	22.38	0.33	-0.01	0.97	0.44	1.06
T12	22.34	0.16	-0.42	0.38	22.36	0.16	-0.01	0.99	0.39	0.94
T13	23.33	0.01	-0.69	0.20	23.78	0.00	-0.42	0.38	0.54	1.31
T14	23.60	0.08	-0.77	0.17	23.87	0.05	-0.45	0.35	0.49	1.18
T15	22.58	0.02	-0.48	0.33	23.28	0.04	-0.28	0.53	0.62	1.50
T16	23.25	0.05	-0.67	0.21	24.46	0.12	-0.62	0.24	0.90	2.19
T17	22.89	0.13	-0.57	0.27	24.16	0.10	-0.54	0.29	0.92	2.24
T18	22.88	0.14	-0.57	0.27	24.16	0.02	-0.54	0.29	0.93	2.25
TH1	22.82	0.11	-0.55	0.28	24.11	0.00	-0.52	0.30	0.93	2.26
TH2	23.39	0.06	-0.71	0.20	23.03	0.12	-0.20	0.63	0.31	0.76
TH3	23.60	0.08	-0.77	0.17	25.33	0.08	-0.88	0.13	1.30	3.1

										6
TH4	22.40	0.08	-0.43	0.37	22.59	0.18	-0.07	0.85	0.43	1.05
TH5	22.72	0.06	-0.52	0.30	25.56	0.07	-0.95	0.11	2.66	6.45
TH6	23.29	0.08	-0.68	0.21	23.81	0.13	-0.43	0.37	0.57	1.37
TH7	23.17	0.09	-0.65	0.23	23.30	0.05	-0.28	0.52	0.43	1.05

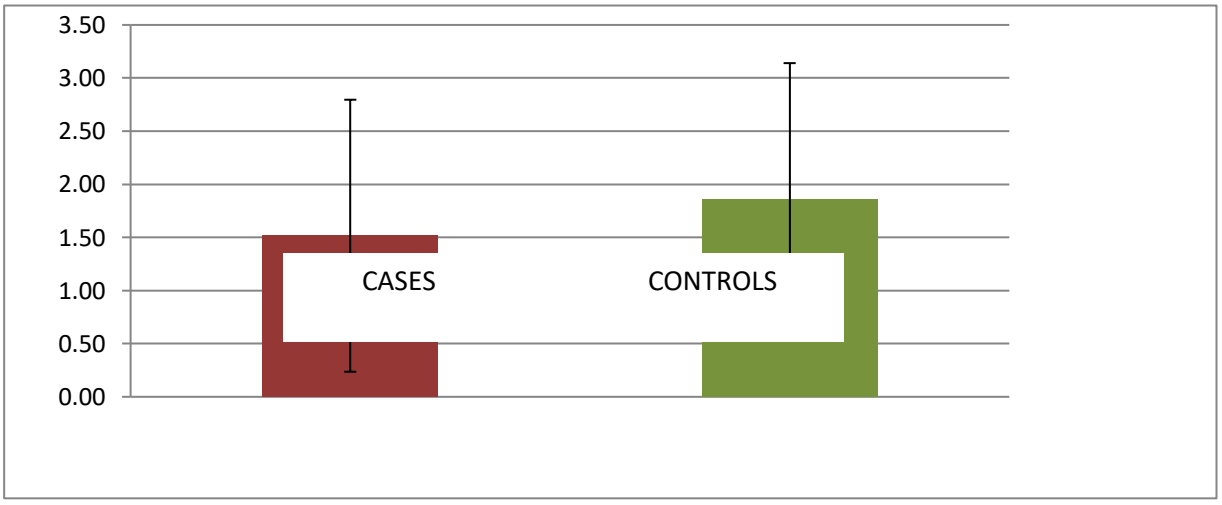
T11	22.17	0.24	-0.37	0.42	22.38	0.33	-0.01	0.97	0.44	1.06
T12	22.34	0.16	-0.42	0.38	22.36	0.16	-0.01	0.99	0.39	0.94
T13	23.33	0.01	-0.69	0.20	23.78	0.00	-0.42	0.38	0.54	1.31
T14	23.60	0.08	-0.77	0.17	23.87	0.05	-0.45	0.35	0.49	1.18
T15	22.58	0.02	-0.48	0.33	23.28	0.04	-0.28	0.53	0.62	1.50
T16	23.25	0.05	-0.67	0.21	24.46	0.12	-0.62	0.24	0.90	2.19
T17	22.89	0.13	-0.57	0.27	24.16	0.10	-0.54	0.29	0.92	2.24
T18	22.88	0.14	-0.57	0.27	24.16	0.02	-0.54	0.29	0.93	2.25
TH1	22.82	0.11	-0.55	0.28	24.11	0.00	-0.52	0.30	0.93	2.26
TH2	23.39	0.06	-0.71	0.20	23.03	0.12	-0.20	0.63	0.31	0.76
TH3	23.60	0.08	-0.77	0.17	25.33	0.08	-0.88	0.13	1.30	3.16
TH4	22.40	0.08	-0.43	0.37	22.59	0.18	-0.07	0.85	0.43	1.05
TH5	22.72	0.06	-0.52	0.30	25.56	0.07	-0.95	0.11	2.66	6.45
TH6	23.29	0.08	-0.68	0.21	23.81	0.13	-0.43	0.37	0.57	1.37
TH7	23.17	0.09	-0.65	0.23	23.30	0.05	-0.28	0.52	0.43	1.05

C1	20.235	0.02	0.16	1.45	20.50	0.141421	0.541459	3.479037	0.416218	1.010
C2	19.83	0.07	0.27	1.87	21.29	0.077782	0.310512	2.044146	0.916007	2.222
C3	21.285	0.08	-0.13	0.74	21.31	0.049497	0.304628	2.016637	0.368755	0.894
C4	21.305	0.05	-0.13	0.73	20.61	0.070711	0.509097	3.229216	0.227382	0.552
C5	20.61	0.07	0.06	1.14	19.83	0.070711	0.738573	5.477386	0.208374	0.505
C6	19.66	0.01	0.32	2.09	20.26	0.035355	0.613538	4.107127	0.507844	1.232
C7	19.93	0.07	0.24	1.76	21.25	0.021213	0.32228	2.100293	0.836696	2.030
C8	20.255	0.04	0.16	1.43	21.88	0.141421	0.135463	1.366037	1.046657	2.539
C9	21.245	0.02	-0.12	0.76	22.31	0.106066	0.010427	1.024301	0.677291	1.643
C10	21.88	0.14	-0.29	0.51	22.17	0.035355	0.051616	1.1262	0.609813	1.479

C11	18.88	0.14	0.53	3.42	19.01	0.09192	0.98129	9.5783	0.84738	2.055
C12	18.605	0.09	0.61	4.07	22.94	0.06364	-0.1749	0.66847	1.0262	2.489
C13	19.255	0.01	0.43	2.7	19.885	0.00707	0.72239	5.27706	2.55409	6.195
C14	20.28	0.01	0.15	1.41	22.94	0.06364	-0.1749	0.66847	2.10521	5.107
C15	20.185	0.01	0.17	1.49	22.605	0.09192	-0.0778	0.83593	1.78811	4.337
C16	19.24	0.01	0.44	2.72	20.28	0.01414	0.60618	4.03816	0.6743	1.636
C17	20.265	0.01	0.15	1.42	21.23	0.01414	0.32669	2.12174	0.6696	1.624
C18	21.23	0.01	-0.11	0.77	21.41	0.06364	0.27521	1.88455	0.40862	0.991
C19	22.305	0.11	-0.41	0.39	22.55	0.14142	-0.0617	0.86766	0.44861	1.088
C20	18.37	0.01	0.67	4.73	19.06	0.02828	0.96511	9.228	0.51254	1.243
C21	19.31	0.04	0.42	2.6	20.19	0.00707	0.63413	4.30658	0.60479	1.467
C22	21.2565	0.03	-0.12	0.76	21.735	0.64347	0.17812	1.50703	0.50246	1.219
C23	22.165	0.04	-0.37	0.43	22.59	0.06364	-0.072	0.84733	0.50206	1.218
C24	21.305	0.12	-	0.73	21.26	0.03323	0.3189	2.08399	0.35234	0.855

			0.13							
C25	20.25	0.03	0.16	1.43	20.31	0.07778	0.59883	3.97034	0.36126	0.876
CONTROL	22.13	0.01	-0.36	0.43	22.26	0.028	0.02	1.06		

Fig:3 Ratio Of Mt Dna Cn Between Test Sample (Gdm) And Contr



Case	Control

Dicussion

In last 25 years, many studies have reported and revealed some changes in mitochondrial function in the pathogenesis of diseases, including T2DM, GDM and ovarian dysfunction etc. The finding that mitochondrial dysfunction and Insulin Resistant can be passed from mother to offspring suggests that mitochondrial dysfunction may be the common underlying defect that links metabolic imbalance with reproductive problems. The relationship between mitochondria and insulin action or ovarian function is highly complex and interdependent. In this study, we have used Real-time PCR to quantify mtDNA copy number in GDM women and control group. Our result indicated that mtDNA copy number of GDM patients was decreased compared to control group. The Mitochondrial dysfunction may induce damage to DNA inside mitochondria which leads to decrease in mitochondrial volume. Our study eventually demonstrated that quantification of mtDNA using real-time PCR is clinically feasible in screening patients with GDM and will further contribute to making an active treatment at the earliest.

References

1. Mitochondrial DNA Copy Number and Developmental Origins of Health and Disease (DOHaD) Hisanori Fukunaga .Int. J. Mol. Sci. 2021, 22, 6634. https://doi.org/10.3390/ijms22126634.
2. Evaluation of mitochondrial DNA copy number estimation ..https://pubmed.ncbi.nlm.nih.gov › .31-Jan-2020
3. Torres JAA. The mitochondrial DNA copy number used as biomarker. Int J Mol Biol Open Access. 2018;3(3):117-119. DOI: 10.15406/ijmboa.2018.03.00063
4. Chunfang Qiu,1 Karin Hevner,1 Dejene Abetew,Mitochondrial DNA Copy Number and Oxidative DNA Damage in Placental Tissues from Gestational Diabetes and Control Pregnancies: A Pilot Study. Published in final edited form as:Clin Lab. 2013; 59(0): 655–660.
5. mtDNA and Mitochondrial Diseases.By: Heidi Chial, Ph.D. (Write Science Right) & Joanna Craig, Ph.D. (Write Science Right) © 2008 Nature Education. Nature Education 1(1):217
6. Kampmann U, Madsen LR, Skajaa GO, Iversen DS, Moeller N, Ovesen P. Gestational diabetes: a clinical update. World J Diabetes. 2015;6(8):1065.
7. Dabelea D, Knowler WC, Pettitt DJ. Effect of diabetes in pregnancy on offspring: follow-up research in the Pima Indians. J Matern Fetal Med. 2000;9:83-88.
8. Buchanan TA, Xiang AH, Page KA. Gestational diabetes mellitus: risks and management during and after pregnancy. Nat Rev Endocrinol. 2012;8(11):639.
9. American Diabetes Association. Standards of medical care in diabetes-2015 abridged for primary care providers. Clin Diabetes 2015;33:97-111
10. He L, Chinnery PF, Durham SE et al (2002) Detection and quantification of mitochondrial DNA deletions in individual cells by real-time PCR. Nucleic Acids Res 30:e68
11. Gahan ME, Miller F, Lewin SR et al (2001) Quantification of mitochondrial DNA in peripheral blood mononuclear cells and subcutaneous fat using real-time polymerase chain reaction. J Clin Virol 22:241–247.
12. Khaled K. Abu-Amero,[1] Thomas M. Bosley,[2,4] and Jose Morales3.Analysis of nuclear and mitochondrial genes in patients with pseudoexfoliation glaucoma. Mol Vis. 2008; 14: 29–36.Published online 2008 Jan 10.
13. ShaleDames∗†Lan-SzuChou‡YeXiao∗TylerWayman∗JenniferStocks∗.The Development of Next-Generation Sequencing Assays for the Mitochondrial Genome and 108 Nuclear Genes Associated with Mitochondrial Disorders.The Journal of Molecular Diagnostics.Volume 15, Issue 4, July 2013, Pages 526-534
14. L.PolimenoaF.CapuanobL.C.MarangiaM.MargiottaaT.Lisowskyc. The augmenter of liver regeneration induces mitochondrial gene expression in rat liver and enhances oxidative phosphorylation capacity of liver mitochondria. Digestive and Liver Disease.Volume 32, Issue 6, August–September 2000, Pages 510-517
15. Harald Niederstätter, Silvano, Köchl .A modular real-time PCR concept for determiningthe quantity and quality of human nuclear and mitochondrial DNA. Forensic Science International: Genetics.Volume 1, Issue 1, March 2007, Pages 29-34

16. Federica Fazzini, Bernd Schöpf, Stefan Coassin.Plasmid-normalized quantification of relative mitochondrial DNA copy number.Scientific Reports volume 8, Article number: 15347 (2018)
17. Jun Yang, Norma Diaz, Joseph Adelsberger.The effects of storage temperature on PBMC gene expression.BMC Immunology volume 17, Article number: 6 (2016)
18. J Huggett, K Dheda, S Bustin & A Zumla Real-time RT-PCR normalisation; strategies and considerations.Genes & Immunity volume 6, pages279–284 (2005)
19. KarstenNielsenaHelle SmidtMogensenaJohannesHedmanbHaraldNiederstätterc Comparison of five DNA quantification methods. Forensic Science International: Genetics,Volume 2, Issue 3, June 2008, Pages 226-230
20. Qian Y. et al. fastMitoCalc: an ultra-fast program to estimate mitochondrial DNA copy number from whole-genome sequences. *Bioinformatics* 33, 1399–1401 (2017). pmid:28453676
21. Knez J. et al. Correlates of Peripheral Blood Mitochondrial DNA Content in a General Population. *Am. J. Epidemiol.* 183, 138–146 (2016). pmid:26702630
22. Tin A. et al. Association between Mitochondrial DNA Copy Number in Peripheral Blood and Incident CKD in the Atherosclerosis Risk in Communities Study. *J. Am. Soc.* Nephrol. JASN 27, 2467–2473 (2016). pmid:26794963
23. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2022). Post-pandemic health and its sustainability: Educational situation. *International Journal of Health Sciences*, *6*(1), i-v. https://doi.org/10.53730/ijhs.v6n1.5949
24. Pyle A. et al. Reduced mitochondrial DNA copy number is a biomarker of Parkinson's disease. *Neurobiol. Aging* 38, 216.e7–216.e10 (2016).
25. Wei W. et al. Mitochondrial DNA point mutations and relative copy number in 1363 disease and control human brains. *Acta Neuropathol. Commun.* 5, (2017).
26. Gómez-Serrano M., Camafeita E., Loureiro M. & Peral B. Mitoproteomics: Tackling Mitochondrial Dysfunction in Human Disease. *Oxid. Med. Cell. Longev.* 2018, (2018).