ORIGINAL ARTICLE
EARLY ONSET DIABETES – GENETIC AND HORMONAL ANALYSIS IN PAKISTANI POPULATION

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Background: Mitochondrial DNA mutation and hormonal imbalance is involved in the pathogenesis of early onset diabetes but data is lacking in Pakistani population. The study was planned to delineate the clinical presentation of early onset diabetes with possible hormonal and genetic etiological factors and aascertain the possible etiological role of insulin and glucagon in these patients either on oral hypoglycaemic or subcutaneous insulin therapy. **Methods:** Retrospective, analytical case control study with conventional sampling technique carried at Centre for Research in Experimental and Applied Medicine (CREAM) affiliated with the department of Biochemistry and Molecular Biology, Army Medical College Rawalpindi from Dec 2006 to July 2011. Study included the patients (20–35 years of age) with early onset diabetes on oral hypoglycemic (n=240), insulin therapy (n=280), and compared with non-diabetic healthy controls (n=150). A fragment surrounding tRNA$^{Leu (UUR)}$ gene was amplified by *AmpliTaq* from mtDNA which was extracted from peripheral blood leucocytes. Then it was subjected to restriction endonucleases, *Apa*I for *A3242G* mutation and *Hae*III for *G3316A* mutation detection. Plasma glucose, glycosylated Hb, osmolality, insulin and glucagon levels along with ABGs analysis was also done. **Results**: Non diabetic controls comprised of 51% males and 49% females, diabetics on oral hypoglycemic 60% males and 40 % females and on insulin therapy 54% males and 46% females. Insulin dependent diabetics had statistically significant hyperglucagonemia, acidemia and bicarbonate deficit. MtDNA *A3242G* and *G3316A* mutations were not detected. **Conclusion:** relative hyperglucagonemia and acidemia in Insulin dependent diabetics was a potent threat leading to DKA. The absence of two mtDNA mutations in ND1 gene rules out the possibility of involvement of these mutations in early onset diabetes in Pakistani population.
Keywords: MtDNA; Mt-ND1 gene; NADH dehydrogenase; Hyperglucagonemia

INTRODUCTION

The worldwide number of patients with early onset diabetes, i.e., 20–30 years of age was projected around 171 million in 2004, predicting to almost double in the year 2030.[1]

Pancreatic beta cells of islets of langerhans are equipped with specialized glucose sensors which are stimulated by hyperglycemia to release insulin which increases hepatic glycogenesis, glycolysis and reduces glucose level in blood. Inadequate insulin secretion, peripheral insulin resistance or both cause diabetes mellitus. Moreover an intricate interaction between genetic mutations and environmental circumstances can also lead to diabetes.[2] DM may be type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes and maturity onset diabetes of young (MODY).[3]

Under normal physiological conditions, pancreatic alpha and beta cells auto regulate secretory activities of each other thus maintaining insulin and glucagon levels in a range required to keep a person normoglycemic. However in diabetes insulin glucagon harmony gets compromised and patient develops relative or absolute hypoinsulinemia along with relative hyperglucagonemia. Initially all drastic features were considered a consequence of hypoinsulinemia but now the role of hyperglucagonemia in diabetic complications has been established. Hyperglucagonemia has more pronounced effect on the plasma levels of glucose, amino acids, fatty acids and ketoacids as compared to insulin deficiency.[4] Hence to minimize the risk of onset of diabetic complications, hyperglucagonemia should be dealt for accordingly.

Isolation and complete sequence analysis of nuclear genome revealed the role of multiple gene mutations in pathogenesis of DM. Sequence analysis of mitochondrial DNA (mtDNA) opened a new window to a sub-class of DM, i.e., "mitochondrial diabetes" with unique maternal inheritance pattern and clinical features like early onset, low BMI, sometimes accompanied with sensorineural auditory deficit and may present as T2D which sooner or later requires insulin therapy. In past few decades the underlying etiological genetic factors have been thoroughly explored and diabetes is now being considered to have strong genetic predisposition.[5]

The role of nuclear DNA mutations in the pathogenesis of type 2 diabetes (T2D) has already

been established as several different DNA mutations have been isolated from these patients like PPARγ, ABCC8, KCNJ11, and CALPN10.[6]

MtDNA is circular, double stranded, having 16569 base pairs (bp) and 37 genes. Out of 37, 22 genes encode tRNAs, responsible for synthesizing mitochondrial proteins, including thirteen polypeptides making integral part of oxidative phosphorylation (OXPHOS), most important being mitochondrially encoded NADH dehydrogenase 1 (Mt-ND1) gene or NADH1 gene.[7] Mt-ND1 gene, 956 base pairs (bp) long (3307-4262 mtDNA bp), is responsible for synthesis of NADH dehydrogenase 1, an integral part of Complex I of mitochondrial electron transport chain generating ATP by oxidative phosphorylation.[8]

Mitochondria DNA is composed of entirely coding DNA and doesn't possess suitable repair system like nuclear genome making all mutations expressive. Moreover continuous generation of reactive oxygen species put mtDNA under oxidative stress. MtDNA mutations disrupt oxidative phosphorylation resulting in over production of reactive oxygen species (ROS) which damage pancreatic beta cells impairing insulin secretion.[9]

The most frequent diabetogenic mtDNA mutation are detected in mtND1 gene and tRNA[Leu], like A3243G, isolated in 1992 and diabetes was called "Maternally Inherited Diabetes & Deafness (MIDD)" due to accompanying sensorineural deafness and maternal inheritance of mitochondrial DNA.[7] Hearing organ "cochlea" and pancreas, being the most active organs, are affected the most when mitochondrial ATP generation is disturbed. Later on G3316A mutation was also isolated as a cause of diabetes.[10,11]

Moreover due to defective mitochondrial activity insulin resistance develops in target tissues leading to hyperglycaemia further deteriorating mitochondrial functional ability. Hence a vicious cycle is set and situation worsens day by day.[12] Mitochondrial diabetes may lead to ECG changes, retinopathies, muscle cramps on exertion and difficulty climbing upstairs, myopathies, proteinuria, which later on develops "focal segmental glomerular sclerosis", renal failure and stroke.[13]

Foregoing in view, it is important that diabetics with symptoms mimicking mitochondrial diabetes should undergo thorough genetic screening, by samples from blood, saliva, muscle biopsies or urine, for accurate diagnosis and treatment. Audiogram can reveal hearing defect at an early stage and can be treated. It is worth mentioning that cochlear implantation has successfully been practiced as remedial measure in mtDNA A3243G mutation since 1990.[14]

Diabetes due to mtDNA mutations is emerging as a one of the most dreadful threats to human health. In Pakistan, researches showing positive relationship between the aetiology of early onset diabetes and mitochondrial DNA mutations are lacking. Keeping in mind the lacuna of available knowledge in Pakistan present study was planned to find out the possible role of genetic factors as well as hormonal imbalance in the pathogenesis of early onset diabetes.

In the light of above, present study was planned to delineate the clinical presentation of early onset diabetes with possible hormonal and genetic etiological factors and aascertain the possible etiological role of insulin and glucagon in these patients either on oral hypoglycemic or subcutaneous insulin therapy. Moreover we tried to find out the prevalence of two diabetogenic mtDNA mutations, i.e., $A2343G$ and $G3316A$ substitution in Pakistani population

MATERIAL AND METHODS

This study was carried out in the Centre for Research in Experimental and Applied Medicine (CREAM) affiliated with the department of Biochemistry and Molecular Biology, Army Medical College Rawalpindi, according to the declaration of Helsinki and approved by the Ethical Committee of the National University of Sciences and Technology, Islamabad. This was retrospective, analytical case control study. Conventional sampling technique was used. Patients were randomly selected from Military Hospital, Rawalpindi, Combined Military Hospital, Multan District and rural areas in Rawalpindi district.

Duration of study was four years. Sample size was 70 and it was divided into three groups, group 1 included non-diabetic healthy persons with no maternal history of diabetes (n=20). Group 2 included patients with early onset diabetes requiring insulin (n=25) and group 3 comprised of patients with early onset diabetes treated by oral hypoglycemics (n=25). Diabetics showing maternal inheritance pattern were included and having paternal history of diabetes were excluded. Diabetics underwent a standard 75g oral glucose tolerance test (OGTT) to confirm the diagnosis according to WHO diagnostic criteria, i.e., fasting blood glucose level ≥ 6.1 mmol/l and random blood glucose level ≥ 11 mmol/l (WHO 1999).[15]

Venous blood samples were analysed for estimation of plasma glucose, glycosylated haemoglobin (Hb_{A1c}), plasma osmolality, plasma insulin and glucagon. Arterial blood gase (ABGs) analysis was also done.

Total genomic DNA was extracted from peripheral blood Leucocytes by Kit method (GENTRA, USA). A standard kit method (QIAGEN

REPLI–g MITOCHONDRIAL DNA KIT)**)** applying isothermal amplification with the help of DNA polymerase was used for the separation and isolation of mtDNA. MtDNA fragment surrounding the tRNA[Leu(UUR)] gene was amplified by polymerase chain reaction (PCR) using forward Primer (5'–CGTTTGTTCAACGATTAAAG–3') covering position 3035 to 3054 and Reverse Primer (5'–AGCGAAGGGTTGTACTAGCC–3') covering position 3437 to 3456. *AmpliTaq* DNA polymerase amplified 422-bp mtDNA fragment containing mtND1 gene (np 3316) and tRNA[Leu].[11] PCR products were electrophoresed on Ethidium Bromide stained 4% agarose gel. These fragments were further subjected to the digestion by restriction endonuclease *Apa*I enzyme for detection of A3242G mutation and *Hae*III restriction endonuclease (New England Biolabs, Beverly and Mass, SA) to detect G3316A mutation.[17] After digestion, DNA was subjected to 2% agarose gel electrophoresis. A3242G mutation creates a recognition site for *Apa*1 restriction endonuclease enzyme cleaving the mtDNA fragment into 208bp and 214 bp fragments. Whereas G3316A mutation was detected by the loss of a *Hae*III restriction site.

PCR restriction fragment length polymorphism (PCR –RFLP) analysis was confirmed by DNA purification followed by desired DNA sequencing process.

Biochemical techniques were used to estimate Plasma glucose (enzymatic colorimetric method using glucose oxidase), HbA$_1$c (ion exchange chromatography), and plasma osmolality (Freezing point depression method). Plasma was separated from venous blood after refrigerated centrifugation at 4–8 °C and stored at -80 °C temperature for estimation of plasma glucagon (Radioimmunoassay (RIA) technique) and insulin (ELISA technique). Partial pressure of carbon dioxide (PCO$_2$) and oxygen (PO$_2$) was determined by Ion selective electrode method.

Bicarbonate ion concentration was calculated by using the measured parameters with Henderson - Hasselbalch equation.[18]

$$HCO_3^- = (\alpha\ PCO_2)\ \text{antilog}\ (pH–pKa)$$

All statistical calculations were done with computer software programme SPSS version 10.00. Data was subsequently examined by One Way ANOVA test. Results are expressed as mean±s.e.m and *p*-value< 0.05 was considered statistically significant

RESULTS

Group-1 comprised of 45% females and 55% males, 52% females and 48% males in group 2 and 59% females and 41% males in group-2. Mean plasma glucose, glycosylated hemoglobin level and plasma osmolality in diabetic patients on oral hypoglycemic drugs (Group-2) and Insulin therapy (Group-3) was significantly higher (*p*<0.001) as compared to controls. Plasma glucagon level was significantly higher in group 2 (*p*<0.05) and group 3 (*p*<0.001) as compared to controls.

Plasma insulin level in group 2 diabetics was significantly higher (*p*<0.05) and significantly lower in group 3 (*p*<0.05) as compared to controls. Plasma osmolality was significantly higher in group 2 diabetics (*p*<0.001) group 3 (*p*<0.05) as compared to controls (Table-1). Arterial blood gases analysis revealed statistically significant acidemia and decreases serum bicarbonate level in group 3 diabetics (*p*<0.05).

We could not identify G3316A as well as A3243G mitochondrial DNA mutations in group 2 and 3 diabetics and normal subjects with no family history of diabetes (Figures-1 to 2). The initial negative results of the less sensitive method, i.e., *Apa*1 and *Hae*III digestion technique, were also confirmed by direct sequencing. One result of group 3 diabetic is shown in figure-3

Table-1: Comparison of plasma glucose, HbA1c, plasma osmolality, plasma insulin and glucagon levels of plasma osmolality of group 2 and 3 with control group.

Group	Plasma glucose (mmol/L)	Glycosylated haemoglobin (%)	Plasma osmolality (mOsmol/Kg of water)	Plasma glucagon (pg / ml)	Plasma insulin (ng/100 ml)
Control (n=20)	5.2±0.2	5.4±0.3	281±1.8	56.5±1.04	56.5±1.7
Diabetics on oral hypoglycaemic drugs (n=25)	16.1±1.1**	7.1±0.2**	296±1.3**	71.23±9.3*	84.9±0.12*
Diabetics on Insulin (n=25)	16.8±0.7**	7.9±0.6**	287±1.6*	90.4±4.28**	39±4.07*

**p<0.001 as compared with normal control subjects (highly significant). *p<0.05 as compared with normal control subjects (significant).

Table-2: Comparison of blood pH, Plasma HCO$_3$, partial pressure of carbon dioxide and oxygen in the ABGs of group 2 and 3 with control group.

| Group	Plasma HCO$_3$ (mmol/L)	PCO$_2$ (mm Hg)	pH	PO$_2$ (mm Hg)
Control (20)	26.7±0.2	38.6±0.823	7.42±0.004	84.6±2.4
Diabetics on oral hypoglycaemic drugs (25)	27.0±0.4	38.3±3.4	7.43±0.01	88.1±9.7
Diabetics on Insulin (25)	24.0±2.2*	37.1±1.786	7.38+0.02*	77.6±4.1

*p<0.05 as compared with normal control subjects (significant)
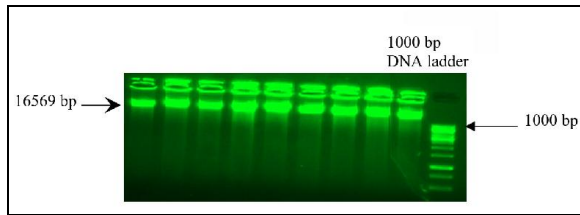
Figure-1: Electropherogram of Ethidium Bromide stained 2 % agarose gel for mitochondrial DNA of the patients with early onset diabetes
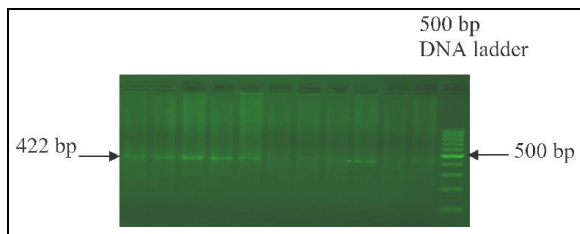
Figure-2: Electropherogram of Ethidium bromide stained 4 % agarose gel for amplified 422 bp segment of mitochondrial DNA in Patients with early onset diabetes
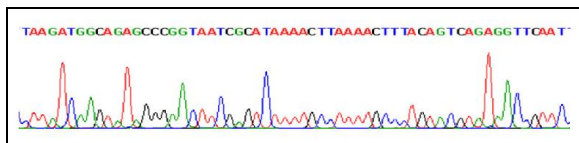
Figure-3: Sequence alignment showing no A3243G and G3316A substitutions in mtDNA of the Patient with early onset diabetes

DISCUSSION

Treatment and rehabilitation of Diabetes, along with its multitude of complications, are casting an increasingly alarming economic burden on health care throughout the world. It has become a painstakingly worrisome task for the under developed and developing countries to meet these challenges with the finite resources at hand, making diabetes a very expensive disorder. Diabetic hormonal imbalance has thoroughly been investigated, and the new era of genetic manipulation and genetic engineering has made genetic analysis of diabetes possible in an easy way. Thorough screening of patients with early onset of diabetes and showing clinical features of mitochondrial DNA mutations involvement as well as maturity onset diabetes (MODY) revealed that such variant of diabetes is very common in Pakistani population but exact aetiology is not clear yet.

Both insulin secretion and insulin sensitivity of target tissues depend on the proper functioning of mitochondrial oxidative phosphorylation.[19] Mitochondrion generates ATP by passing electrons from protein complexes of low redox potential to the ones with higher redox potential and thus, creating potential difference across inner mitochondrial membrane which is later used in ATP synthesis. Being the distinctive cellular organelles, mitochondria play integral role in metabolism of carbohydrates and fatty acids and in biological oxidation. Pancreas is affected the most by mtDNA mutations.[20] Foregoing the association of early onset diabetes and mtDNA mutations, present study was focused to identify two of the frequently detected diabetogenic A3243G and G3316A mutations in mtND1 gene and associated region respectively. Early onset diabetes usually shows other mutations in mtND1 gene as well like T3394C, A3426G, C3497T.[21] The mtND1 gene is responsible for polypeptides making complex 1 of electron transport chain, i.e., NADH Dehydrogenase complex. Thus, any of these mutations will disrupt the process of mitochondrial oxidative phosphorylation. Therefore, secretion of insulin from pancreatic beta cells gets impaired, leading to development of diabetes. In present study, genetic screening showed the absence of two common diabetogenic mutations (A3243G and G3316A) in mitochondrial DNA. However the possibility of mtDNA mutation cannot be excluded as there are many other nucleotide substitutions which may be the leading cause of diabetes. Unfortunately due to lack of funds and resources limited number of patients and one mitochondrial gene was analyzed.

Significant Hypoinsulinemia was detected in patients on insulin therapy indicating dysfunction or loss of beta cells of pancreatic islands to produce sufficient insulin. Whereas patients treated with oral hypoglycaemia presented with statistically significant hyperinsulinemia reflecting the peripheral insulin resistance as main etiological factor.[22]

As in selected group of early onset diabetes both variant were observed, one with pancreatic insulin insufficiency and other with insulin resistance, hence it can be concluded safely that early onset diabetes is not a typical feature of type 1 diabetes but aetiology involves other factors too.

It is a potent threat that can deteriorate diabetes more than defective insulin production. Catabolic effects of glucagon lead to uncontrolled hyperglycaemia, protein catabolism, raised free fatty acid level in blood and marked ketosis. Usually this parameter in ignored but this study revealed that accompanying hyperglucagonemia silently damages the situation and give rise to diabetic complications including microvasculature, neurological abnormalities, renal dysfunction and ocular issues. Diabetics have to face serious implication regarding micro and macrovascular complications and their management.[23] These complications make the life of diabetic much miserable leading to disability, amputation and even death at a younger age.[24]

Marked hyperglucagonemia in patients with reduced plasma insulin level strengthens the fact that insulin released in response to hyperglycemia promotes insulin release which in turn acts on insulin receptors located on pancreatic alpha cells. Insulin binding activates ATP sensitive potassium channels leading to hyper polarization of alpha cells. Unlike beta cells, alpha cells production of glucagon is suppressed. Unfortunately in diabetes with hypoinsulinemia, glucagon secretion is unchecked and its level in blood rises. [25]

Patients requiring insulin therapy showed statistically significant acidemia and it is quite in relation with hyperglucagonemia in the same group. Glucagon being ketogenic in nature, tends to raise plasma levels of ketoacids particularly hydroxybutyrate.[25] Patients having statistically significant hypoinsulinemia and hyperglucagonemia also had blood pH though normal but closer to lower limit (normal 7.35–7.45) as well as reduced serum bicarbonate ions. Hence it can be concluded that slight overproduction of ketoacids, beta hydroxy butyrate and acetoacetate, replacing serum bicarbonate ions. These finding show that these insulin dependent diabetics have the tendency to develop DKA if mentioned disturbances remain uncompensated.[22]

All diabetics showed statistically significant hyperglycaemia due to enhanced gluconeogenesis, glycogenolysis and decreased peripheral glucose utilization. Hyperglycaemia along with raised glycosylated haemoglobin level is not only reflecting the effects of hypoinsulinemia and hyperglucagonemia but indicating poor diabetic control. This may be attributed to the fact that most of the patients belonged to rural areas and were illiterate so they didn't realize the significance of glycaemia control during DM. Plasma Hyperosmolality was a strong warning of subsequent diabetic complications like hyperosmolar non ketotic coma and diabetic acidosis (DKA) – acute metabolic complications requiring urgent management.[24,25]

In present study, all families were selected precisely on the basis of maternal inheritance pattern of diabetes. Moreover it was noticed that clinical presentation and phenotype of diabetics from selected families was mostly according to the cases already reported internationally, like the onset of diabetes at young age, the early requirement of insulin therapy due to ineffectiveness of oral hypoglycemic agents, low body mass index, and impaired hearing in most of the cases. In addition statistically significant findings in diabetics on insulin therapy indicate their tendency to develop DKA if not treated well in time.

CONCLUSION

As association of mtDNA and diabetes is getting stronger day by day so absence of mtDNA tRNA[Leu] gene and NHDH dehydrogenase mutations in study sample does not rule out their significance. Hypoinsulinemia, hyperglucagonemia and acidemia are alarming features in insulin dependent diabetics and must be addressed to avoid development of diabetic complications and rehabilitate these patients, no matter whatever pathophysiology involved in early onset diabetes.

RECOMMENDATION

Advanced research trials on mtDNA gene mutations with larger sample size and more extensive mtDNA sequencing, environmental and hereditary factors and hormonal interplay, are required to find out the possible explanation for early onset diabetes to determine genotype – phenotype correlation for the mentioned disease in Pakistani population.

Conflict of interest declaration: No competing interests are involved in this study.

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AUTHOR'S CONTRIBUTION

This project required extensive and selective sampling, appropriate storage and then molecular as well as biochemical analysis. Principal author performed all these tasks. Second author helped in selection of some required patients and sampling from remote rural areas.

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of Diabetes estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27(5):1047–53.
2. Devendra D, Liu E, Eisenbarth GS. Type 2 diabetes: recent developments. BMJ 2004;328(7442):750–4.
3. Bhatia S, Rachna, Bansal P, Mani S. Mitochondrial diabetes: Differential diagnosis, features, and its possible management. JISMA 2014;27(4):213–5.
4. Walker JN, Ramracheya R, Zhang Q, Johnson PR, Braun M, Rorsman P. Regulation of glucagon secretion by glucose: paracrine, intrinsic or both? Diabetes Obes Metab 2011;13(Suppl 1):95–105.
5. Lehto M, Wipemo C, Ivarsson SA, Lindgren C, Lipsanen-Nyman M, Weng J, et al. High frequency of mutations in MODY and mitochondrial genes in Scandinavian patients with familial early onset diabetes. Diabetologia 1999;42(9):1131–7.
6. Pearson ER, Badman MK, Lockwood CR, Clark PM, Ellard S, Bingham C, et al. Contrasting diabetes phenotypes associated with hepatocyte nuclear factor – 1alpha and – 1beta mutations. Diabetes Care 2004;27(5):1102–7.

7.   Starkov AA. The role of mitochondria in reactive oxygen species metabolism and signaling. Ann. N Y Acad Sci 2008;1147:37–52.

8.   Tuppen HA, Blackely EL, Turnbull DM, Taylor RW. Mitochondrial DNA mutations and human disease. Biochim Biophys Acta 2010;1797(2):113–28.

9.   Patti ME, Corvera S. The role of mitochondria in the pathogenesis of type 2 diabetes. Endocr Rev 2010;31(3):364–95.

10. Dongre UJ, Meshram VG. Is mitochondrial DNA responsible for maternally inherited type 2 diabetes mellitus? A hypothetical review. Int J Pharm Sci Rev 2014;28(1):179–87.

11. Su SL, Kou CL, Liu CH. Diabetes and mitochondria. Chang J Med 2013;11(1):1–7.

12. Iwasawa R, Mahul Mellier AL, Datler C, Pazarentzos E, Grimm S. Fis1 and Bap31 bridge the mitochondria – ER interface to establish a platform for apoptosis induction. EMBO J 2011;30(3):556–68.

13. Chen YN, Liou CW, Huang CC, Lin TK, Wei YH. Maternally inherited diabetes and deafness (MIDD) syndrome: A clinical and molecular genetic study of Taiwanese family. Chang Gung Med J 2004;27(1):66–72.

14. Rosenthal EL, Kilney PR, Boerst A, Telian SA. Successful cochlear implantation in a patient with MELAS syndrome. Am J Otol 1999;20(2):187–91.

15. Assal J, Groop L. Definition, diagnosis and classification of diabetes mellitus and its complications. World Health Organ. 1999;1–65.

16. Ohkubo K, Yamano A, Nagashima M, Mori Y, Anzai K, Akehi Y, et al. Mitochondrial gene mutations in the tRNALeu(UUR) region and diabetes: prevalence and clinical phenotypes in Japan. Clin Chem 2001;47(9):1641–8.

17. Pranoto A. The association of mitochondrial DNA mutation G3316A and T3394C with diabetes mellitus. Folia Medica Indones 2005;41(1):3–8.

18. Morris CJOR, Morris P. Separation methods in biochemistry. London. Pitman Press 1976;2:93–148.

19. Achilli A, Olivieri A, Pala M, Hooshiar Kashani B, Carossa V, Perego UA, et al. Mitochondrial DNA backgrounds might modulate diabetes complications rather than T2DM as a whole. PLoS One 2011;6(6):e21029.

20. Wang S, Wu S, Zheng T, Yang Z, Ma X, Jia W, et al. Mitochondrial DNA mutations in diabetes mellitus patients in Chinese Han population. Gene 2013;531(2):472–5.

21. Yusnita Y, Norish D, Rahman AJ. Mutations in mitochondrial NADH dehydrogenase subunit 1 (mtND1) gene in colorectal carcinoma. Malays J Pathol 2010;32(2):103–110.

22. Bansal P, Wang Q. Insulin as a physiological modulator of glucagon secretion. Am J Physiol Endocrinol Metab 2008;295(4):751–61.

23. Piero MN, Nzaro GM, Njagi JM. Diabetes mellitus–a devastating metabolic disorder. Asian J Biomed Pharm Sci 2014;4(40):1–7.

24. Godoy-Matos AF. The role of glucagon on type 2 diabetes at a glance. Diabetol Metab Syndr 2014;6(1):91–5.

25. Gromada J, Franklin I, Wollheim CB. Alpha cells of the endocrine pancreas: 35 years of research but the enigma remains. Endocr Rev 2007;28(1):84–116.

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