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A study on variation of DNA concentration in Diabetes affected people of Andhra Pradesh State, India

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ABSTRACT

Diabetes is a metabolic disorder in which blood glucose levels are too high due to either lack of enough insulin or the body cannot respond to the insulin produced by pancreas. Worldwide there are 387 million diabetes people. Certain genetic and environmental factors contribute to development of diabetes. Changes in DNA and its specific gene with a small effect alter the cell function and its response is associated with many diseases including diabetes. There is increasing evidence that number of deaths in diabetes subjects are due to high temperatures and humidity. Diabetic subjects would be affected by these changes with the change in environment with the change in temperature. This study focuses on the metabolic changes that affect diabetes in aged subjects. This work provides the observations which show the levels of variation in DNA which were treated at different temperatures within different age groups of individuals.

KEY WORDS: Diabetes, Temperature, DNA, BDNF.

1. INTRODUCTION

According to WHO (World Health Organisation) status, Diabetes is one of the leading causes of deaths across the world. Globally there are 387 million peoples suffering for this disease, 4.9 million deaths and 66.8 million suffering for diabetes in every year having age group of 20-79 years (International Diabetes Federation). In India about 63 million peoples are suffering with this disease (www.diabetesfoundationindia.org) basically there are two types of diabetes i.e. Type1, Type II and many other. Type 1 diabetes, the immune system attacks insulin producing pancreatic beta-cells and destroys. In this case diabetes people mainly depend on external insulin source. Type II diabetes, insulin is produced by beta-cells but the body becomes less responsive to it (Jerrold et al.,).The highest numbers of diabetes peoples are between 40-59 years of age (International Diabetes Federation) in 2014 (Maria et al., 2006). Approximately half of the cases with diabetes are undiagnosed until they develop other health complications. Some of them are Diabetic Neuropathy, Nephropathy, Retinopathy, peripheral arterial disease, Psychiatric disorders, malignancy, pulmonary diseases, sleep disorders, and hearing impairment (Jeff, 2013; WHO/NCD/NCS/99.2).

Diabetes is mainly caused by genetic and environmental factors (WHO,). Certain variants like HLA genes are important genetic risk factors for type 1 diabetes. These genes benefit in producing proteins that participate in immune reactions (Laura, 2004). According to Diabetes Prevention Program, variants of TCF7L2 gene increase the chance of affecting type II diabetes. There are many other genes involved in the pathogenesis of disease. Difficulties in the binding of insulin and its receptor can lead to diabetes (Grant, 2009; Diabetes Prevention Program Research Group) Many subjects have little DNA variants that affect gene expression in the beta-cells of pancreas. This enables us to know the reason which causes a change in exact DNA variant inducing diabetes (Sam, 2014)

DNA of each human cell is exposed to damage naturally and by environmental factors comprising ionising radiation, UV rays, diet and chemicals. There are various environmental factors, among which temperature is the most common factor in affecting DNA. Diabetes peoples undertaking mechanical work in the high temperature or with exposure to high temperature and high moisture could be extremely wane. Mostly people with diabetes are hospitalized during extreme high temperatures. These conditions get worse in some cases and progressively lead to death. High temperature lessens glucose levels due to enlargement of the blood vessels. So this makes the glucose metabolic rate faster than normal (Serge, 2008). Characterisation of person's age with respect to temperature has a considerable effect on the individual's DNA. Few statistical data suggest that aged persons are at greater risk of exhibiting heat stress. Ageing is coupled with diminished heat tolerance and changes in responsesof heat regulation. Finally ageing is interconnected with diabetes which further influences heat tolerance and heat regulation (Kundu, 2013). Few readings also suggest that at higher temperatures fraying effect is observed within the oligomers in DNA which results in genetic variations (Kenny, 2012).

2. MATERIALS AND METHODS

Blood samples were obtained from both control and diabetic people of different age groups. Blood samples were collected from subjects and stored in K3 EDTA vials. Blood was allowed to settle through which plasma and

whole blood was separated. Those separations were stored separately; whole blood was stored in same K3 EDTA vials at -20^{0}C. Diabetic samples were obtained from those individuals who were already diagnosed by the disease and were under prescription. Persons were selected over the age of 40-60 years and divided them under three age groups. The S1, S2 of all the figures indicates age 40, S3, S4 indicates age 50 and S5, S6 indicates 60 respectively. The figure.1 and figure.i, indicates the blood samples of control and diabetic incubated at 35°C temperature; figure.2, & figure.ii, indicates the blood samples of control and diabetic incubated at 55°C temperature; figure.3 and figure.iii, indicates the blood samples of control and diabetic incubated at 60°C temperature; figure.4, and figure.iv, indicates the blood samples of control and diabetic incubated at 80°C temperature; figure.5 & figure.v, indicate the blood samples of control and diabetic incubated at 98°C temperature respectively for 5 hours each. After the incubation process, DNA extractionwas performed for the temperature affected blood samplesby non-enzymatic salting out method (Sajja, 2014). Fromthis method large quantity of DNA was obtained through series of RBC lysis followed by cell lysis and precipitation of DNA. To check the DNA, electrophoresis was done using 0.8% agarose gels and visualized under gel doc. DNA quantification was done using UV visible spectrophotometer.

3. RESULTS

Blood samples of control and diabetic subjects were 1st treated with 35°C (figure.1) by incubating 100ul blood for 3-4 hours from which DNA was later isolated. 35°C specific temperature was selected as this temperature is the general maintained temperature at the region, East Godavari district; Andhra Pradesh, where the samples were collected. Next same blood samples were treated at 55°C and 60°C (figure.2 and 3 respectively), these temperatures are the ideal thermo cycler temperatures which are mostly considered annealing temperatures which could show the stability of DNA at these temperatures. Then to observe the DNA variation at higher temperatures, temperatures were gradually increased to 80°C and 98°C (figure.4 and 5 respectively) and same blood samples were treated at these temperatures. To observe the gradual variation in DNA, table.1 and 2 represents the samples of male and female subjects respectively which were incubated at variable temperatures. For the isolated DNA samples, DNA quantification was done through spectrophotometric analysis.

Control samples:

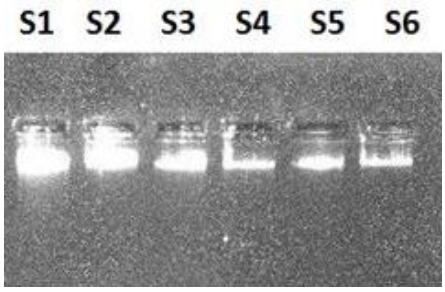

Figure.1. Agarose gel sample treated at temperature 35°C

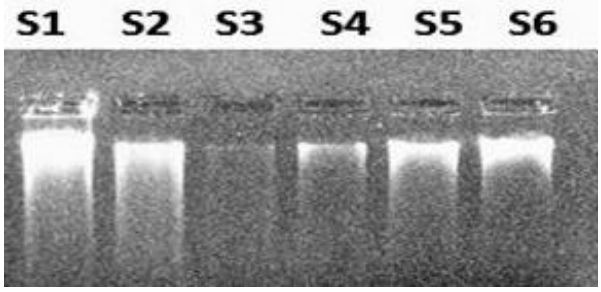

Figure.2. Agarose gel sample treated at temperature 55°C

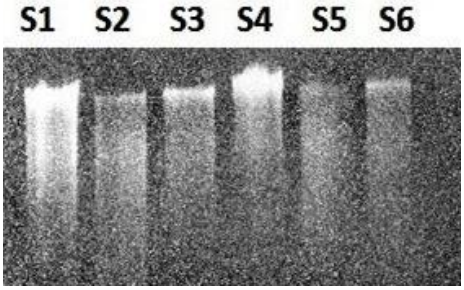

Figure.3. Agarose gel sample treated at temperature 60°C

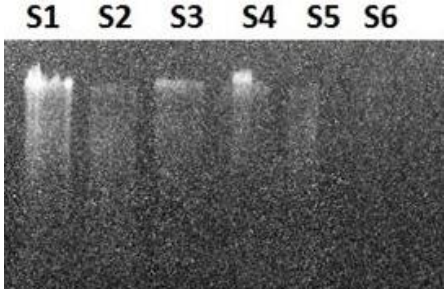

Figure.4. Agarose gel sample treated at temperature 80°C

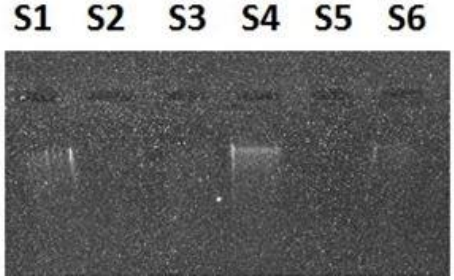

Figure.5. Agarose gel sample treated at temperature 98°C

Diabetic Samples:

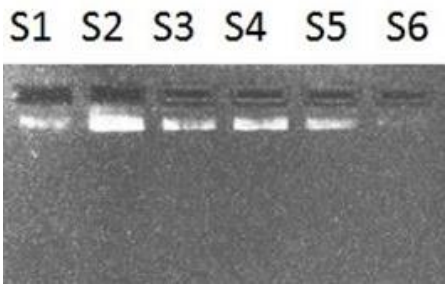

Figure.i. Agarose gel of diabetic sample incubated at temperature 35°C

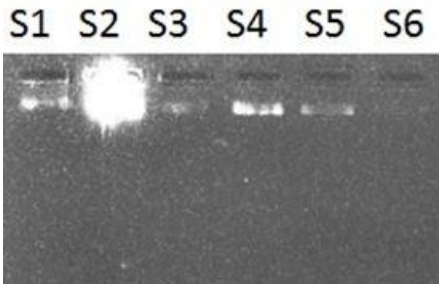

Figure.ii. Agarose gel of diabetic sample incubated at temperature 55°C

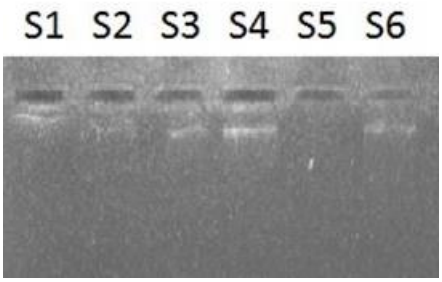

Figure.iii. Agarose gel of blood sample of diabetics incubated at temperature 60°C

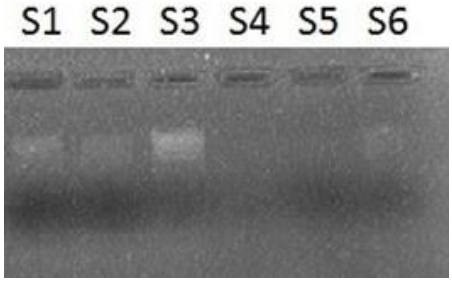

Figure.iv. Agarose gel of blood sample of diabetics incubated at temperature 80°C

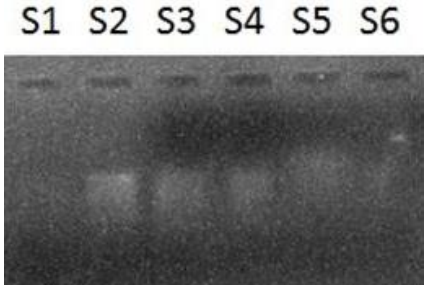

Figure.v. Agarose gel of blood sample of diabetics incubated at temperature 98°C

Results tables:

Table.1. Control subjects of various age groups and gender at different temperatures

Sample	Sex	Age	Incubation Temperature					Concentration(µg/ml)				
								35^0C	55^0C	60^0C	80^0C	98^0C
S1	M	40	35^0C	55^0C	60^0C	80^0C	98^0C	78.2	82.3	71.1	64.9	28.4
S2	F	40	35^0C	55^0C	60^0C	80^0C	98^0C	77.6	73.7	61.4	50.2	9.8
S3	M	50	35^0C	55^0C	60^0C	80^0C	98^0C	71.8	56.6	65.3	57.4	20.6
S4	F	50	35^0C	55^0C	60^0C	80^0C	98^0C	70.5	64.5	69.6	58.9	36.7
S5	M	60	35^0C	55^0C	60^0C	80^0C	98^0C	72.1	70.8	58.1	47.5	19.3
S6	F	60	35^0C	55^0C	60^0C	80^0C	98^0C	69.7	72.8	60.4	20.4	25.9

Table.2. Diabetic subjects of various age groups and gender at different temperatures

Table i												
Sample	Sex	Age	Incubation Temperature					Concentration(µg/ml)				
								35^0C	55^0C	60^0C	80^0C	98^0C
S1	M	40	35^0C	55^0C	60^0C	80^0C	98^0C	79.5	83.7	79.4	69.3	28.4
S2	F	40	35^0C	55^0C	60^0C	80^0C	98^0C	90.5	90.4	69.0	53.6	22.6
S3	M	50	35^0C	55^0C	60^0C	80^0C	98^0C	75.8	59.2	67.8	57.4	18.7
S4	F	50	35^0C	55^0C	60^0C	80^0C	98^0C	79.5	85.4	75.6	45.6	16.4
S5	M	60	35^0C	55^0C	60^0C	80^0C	98^0C	76.3	70.1	59.9	41.2	13.8
S6	F	60	35^0C	55^0C	60^0C	80^0C	98^0C	70.9	72.9	76.6	31.3	12.7

Among 40-60 years subjects with diabetes, the amount of DNA extracted was reduced as the blood samples were exposed to higher temperatures. Good amount of DNA was obtained when incubated at the lower temperature 35°C for 5 hours (figure.1) with a slight decrease in amounts of DNA followed by incubation at higher temperatures 55°C, 60°C, 80°C and 98°C.

4. DISCUSSION & CONCLUSION

In the olden days, the term protein deficiency was very common and the people were not aware of the RDA (Recommended Dietary Allowance) but gradually the people became conscious by the awareness programs conducted by the government. Besides this achievement, the protein deficiency is being rooted with the increasing environmental effects (Temperature).

Andhra Pradesh a state in India, has a Tropical semi-arid (steppe) climate. The temperature is gradually increasing annually. The highest temperature recently recorded in the May-June 2015 was 49^0C and the temperature was expected to be increased by 0.2-1^0C annually. This increase is due to the increase in global warming.

In Diabetic patients, glucose metabolism is disturbed. When such persons are subjected to high temperatures, variation is observed with in the nucleotides of DNA. This variation can be caused by few effects like the fraying in the oligos within the DNA. These effects on DNA would cause variations in further processing of proteins and finally leads to disturbances in various metabolisms which would cause changes in genome which are expressed in insulin-responsive tissues that are regulated by insulin. These causes various disorders associated with aging process which is commonly observed in diabetic patients.

In our research shows that Diabetic DNA's showed denaturation when compared with the controls and quantity was gradually reduced when exposed to higher temperature. This shows that diabetic subjects are sensitive to higher temperatures.

5. ACKNOWLEDGEMENT

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