DNA Damage Observed in Unaffected Individuals with Family History of T2DM

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Abstract: Diabetes has been documented to cause high levels of DNA fragmentation in some cases. As diabetes is inheritable and influenced by both genetic and environmental factors, an investigation into the genomic stability of individuals who are strongly at risk of inheriting diabetes was conducted by inducing oxidative stress, as DNA damage in unaffected individuals could be a sign of onset of the disease or the presence of genetic alterations that reduce cellular defences against reactive oxygen species. In this study, alkaline comet assay was performed on isolated human leukocytes to determine whether individuals with a family history of Type 2 Diabetes Mellitus (T2DM) are more prone to DNA damage under oxidative stress. Visual scoring of comets showed that these individuals have higher degree of DNA damage compared to a control individual with no family history of Type 2 Diabetes Mellitus. Further studies with large sample could determine the presence of disabled cellular defences against oxidative stress in unaffected individuals and intervention with antioxidants could prevent or manage Type 2 Diabetes Mellitus and its complications.

1. Introduction
Type II Diabetes Mellitus is a type of metabolic disorder that causes higher blood sugar levels than normal due to insulin resistance. High blood sugar levels cause the formation of reactive oxygen species that cause DNA damage by disabling the cellular defences against oxidative stress. These in turn cause the many complications of diabetes such as ketoacidosis (abnormally high production of ketone bodies which results in alter of blood pH), nephropathy, retinopathy etc. These complications seem to be the result of activation and alteration of various cell signalling pathways, particularly apoptotic pathways, by the free radicals/ reactive oxygen species. NF-κB, VEGF, JNK/SAPK and p38 MAPK pathways have been implicated in the same.

While the presence and effects of DNA damage due to oxidative stress induced by the free radicals have been long observed, pre-symptomatic testing for the same in individuals who are susceptible to T2DM has not yet been investigated. Given that diabetes is highly inheritable and that epigenetic mechanisms play an important role in the same, it is likely that at-risk individuals (with a strong family history of diabetes) may have a higher degree of DNA damage than normal even if they have not developed the disease yet. If DNA damage is indeed observed in such individuals, they may take antioxidants as a preventative measure against the complications of diabetes. This may also have implications on their fertility as sperms of diabetic individuals have been observed to show DNA damage. Fertility issues of at risk individuals and statistics of children born with genetic abnormalities to diabetic parents have not been investigated yet.

This project will check for the amount of DNA damage in individuals with high risk of developing inherited diabetes, defined as with 4 or more immediate members in the family suffering from Type 2 Diabetes Mellitus for purposes of this experiment, by alkaline comet assay.
2. Materials and Methods
Candidates were selected based on family history of Type 2 Diabetes Mellitus. Three females with four or more immediate family members diagnosed with T2DM were selected for this project. Another female of same age with no immediate family members with T2DM was selected as control. BMI, diet, exercise patterns and pedigree charts were compiled for each individual.

2.1 Leukocyte Isolation: Leukocytes were isolated from whole blood samples of the selected individuals using Histopaque 1077 medium by density gradient centrifugation [3]. The protocol for alkaline comet assay was adapted from the protocols described by Olive and Banáth [11] and Dhawan et al [16]. For induction of oxidative stress, H$_2$O$_2$ values were referenced from a study on effect of exercise on biomarkers of DNA damage in individuals with T2DM [4].

2.2 Alkaline Comet Assay: Agarose coated slides were prepared with normal melting point agarose. 50 µL of leukocytes isolate was cultured with RPMI 1640 media along with FBS for 24 hours. The cultures were divided into 2 batches- A (without H$_2$O$_2$) and B (with H$_2$O$_2$). After 24 hours, 100µM of H$_2$O$_2$ was added to batch B to induce oxidative stress. At the end of peroxide treatment, the cells were suspended in 0.5% LMPA (Low Melting Point Agarose) dissolved in PBS. The mixture was cast on pre-coated agarose slides, covered with coverslip and labelled before treatment with lysing solution. The slides were washed with PBS and kept in electrophoresis chamber. Current was run at 25V for 20 minutes. After neutralisation in neutralisation buffer, the slides were stained with Ethidium Bromide and observed under fluorescent microscope. The comets were visually scored at random for extent of DNA damage.

3. Results
Visual scoring of the slides was done and the cells were classified based on length of the comet tails as per the protocol showed that there were no comets in batch without peroxide treatment in both control and test slides while the batch subjected to peroxide treatment showed varying amount of class 1 and class 2 comets in test slides but not the control slide.

For Test individual T1, 44% of the observed cells were Class 0 or cells with no significant observable DNA fragmentation. However, a small amount of T1’s cells (6%), were Class 1 comets. Test individual T2 also had a large fraction of Class 0 comets (34%), however, they also had 12% and 2% of Class 1 and Class 2 comets respectively. Test individual T3 had the most number of Class 1 and Class 2 comets at 18% and 10% respectively. This may be significant as this individual had the most number of immediate affected relatives, as depicted in Figure 1.

![Figure 1. Pedigree of Test Individual 3](image-url)
None of the samples had comets with very high degree of DNA fragmentation i.e. Class 3 and Class 4 comets were absent.

4. Discussion
The causative factors of T2DM are both genetic and environmental and function in an obscure manner for development and progression of the disorder. The Comet assay is sensitive for DNA lesions caused by free radicals. DNA damage due to oxidative stress in diabetic individuals has been reported by many researchers [6], [8]. The free radicals produced by hyperglycaemia increase cellular and DNA damage; a side effect which is the alteration of apoptotic mechanisms that push cells to the point of no return [7]. This results in the development of nephropathy, angiopathy and other complications associated with diabetes [5]. DNA damage in individuals with a strong family history of diabetes who have not yet been diagnosed with the same shows that they may susceptible to the above complications. Apart from an active lifestyle which is commonly recommended by physicians to such individuals; increased intake of antioxidants may also be suggested as in this case, the individual with lowest BMI had the most comets. This project is a preliminary investigation. Incorporation of other factors such as blood sugar levels logs, apoptosis studies, DNA damage in other cells and tissues such as sperm which have been proven to show higher degree of DNA damage in diabetic individuals can be checked as it may have implications on fertility and genetic stability of children fathered by diabetic fathers [2]. Inclusion of antioxidants in diet and studying the effects of the same on DNA damage levels by various antioxidant assays may be undertaken to establish the presence of DNA damage in individuals with inherited susceptibility to T2DM.

5. Conclusion
DNA damage was observed in the leukocytes of individuals with strong family history of T2DM. This may be due to intrinsic genetic factors that disable cellular defences against oxidative stress. If established by further studies, those with strong family history of T2DM may take antioxidants to prevent complications of diabetes.

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