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Research Article

# ASSESSMENT OF THE METABOLIC PROFILE IN PATIENTS OF TYPE 2 DIABETES MELLITUS AND HYPOTHYROIDISM THROUGH COMET ASSAY

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**ABSTRACT**

**Objective:** To determine the extent of cell damage in the patients of type 2 diabetes and hypothyroidism subjects with the help of comet assay.
**Design**: Cross-sectional analytical study.
**Place and Duration of study:** Gujarat Endocrine Centre and National Institute of occupational health (NIOH) from January 2009 to December 2009.
**Patients and methods:** Blood samples were collected from 50 subjects [Control; diabetic subjects receiving oral hypoglycemic agents (DM-OHA) and Insulin (DM-I); hypothyroid (HT) subjects; and subjects suffering from both diabetes and hypothyroid (HT+DM). n=10 for each group]. Peripheral blood samples were collected, embedded in agarose gel, lysed in high ionic strength solution with Triton X-100, and then electrophoresed at pH>13. Ethidium bromide stained slides were then subjected to analysis under fluorescent microscope.
**Results:** Subjects of HT did not show any remarkable cell damage as compared to control. Extent of cell damage in diabetic subjects and subjects with both metabolic disorders was significantly high ($p<0.05$). It is also evident from quantitative comet metrics namely tail length, tail DNA and olive tail moment (OTM). Correlating the OTM with HbA1c ($R^2=0.98$) and BMI ($R^2=0.91$) showed a good correlation. The subjects receiving sulphonamides as a therapeutic treatment for the diabetes control reflected high OTM values.
**Conclusion:** Unlike hypothyroidism, diabetes mellitus may be recognized as an indicator of cell damage and is well correlated with cellular damage parameters.

**Key words:** Type2 diabetes mellitus, Hypothyroidism, Comet Assay, Olive Tail Moment.

## INTRODUCTION

Type 2 diabetes mellitus (T2DM) is described as an endocrine disorder involving the secretion of insulin and its action on target tissues[1]. It has been one of the most urgent medical problems of 21st century in view of epidemic proportions it has attained[2]. Thyroid disorders are also getting the increased clinical attention in last decades. A number of reports have also indicated a higher than normal prevalence of thyroid disorders in type 2 diabetes mellitus (T2DM) patients, with hypothyroid being the most common disease[3]. Mechanisms like metabolic stress resulting from changes in energy metabolism, the levels of inflammatory mediators and the status of antioxidant defense may contribute to cellular DNA instability. Also, remarkable abnormalities in the physical properties of erythrocytes from diabetic patients have been reported[4].

The single-cell gel electrophoresis technique or comet assay is widely regarded as a quick and reliable method of analyzing DNA damage in individual cells[5]. DNA strand breaks allow DNA to extend from lysed and salt-extracted nuclei, or nucleoids, to form a comet-like tail on alkaline electrophoresis. Cells undergoing active cell death or apoptosis demonstrate highly fragmented DNA. Progression of cell death results in the extensive formation of double-strand breaks and is readily detected using alkaline electrophoretic conditions. To this moment, no data have been published concerning assessment of cellular DNA instability in T2DM patients having hypothyroid co-existence using the comet assay. Therefore, the objectives of the current study are as shown below,

(1) The aim of this work was to study qualitatively the development of comet images evaluating extent of cellular damage and quantify different comet metrics.In addition, to establish correlation of different measurements in the comet assay.

(2) To establish the relationship between thyroid dysfunctioning and T2DM using thyroid peroxidase antibody (TPO), glycated haemoglobin (HbA1c) and body mass index, BMI (Quetelet index) measurement.

(3) To evaluate the effect of drug treatment on cell damage.

## PATIENTS AND METHODS

### Subject selection and collection of blood samples

DNA damage is an indication of cellular damage. Comet assay is widely regarded as a quick and reliable method for analyzing DNA damage in individual cells. Two ml of venous blood samples were collected in heparinised vials. Subjects of two different metablic disorders namely type 2 diabetes mellitus (T2DM) and/or hypothyroidism was recruited in the present study.

Human ethical committee of L.M. College of Pharmacy, Ahmedabad, approved the protocol for the present study. All the patients were given verbal and written information about the study prior to providing written consent and invited for verbal and written feedback of individual results at the end of the study. Hypothyroid subjects (HT), diabetic subjects receiving oral hypoglycemic agents therapy (DM-OHA), diabetic subjects receiving Insulin therapy (DM-I) and subjects with diabetes and hypothyroidism coexistence (HT+DM) were recruited into the present study (n=10 for each group). All the study groups were compared with the 10 control subjects.

Subjects with hypothyroidism received levothyroxine as a hormone replacement supplement. Subjects with diabetes received oral hypoglycemic agents and/or Insulin to control blood sugar levels. 90 % of DM-OHA population received glibenclamide, and 10 % population received gliclazide monotherapy. HT+DM subjects received glibenclamide in combination with levothyroxine treatment.

### Chemicals Purchase

Low melting agarose, normal melting agarose, triton X-100 and phosphate buffer saline (PBS) ($Ca^{++}$, $Mg^{++}$ free) were purchased from HiMedia pvt. laboratories (Mumbai). All other chemicals were of the highest purity available.

### Methodology

Half frosted slides (Blue star, Mumbai, India) were dipped into a chromic acid solution and then 100 % methanol to remove particulate matters. Half-frosted slides were dipped into 1% normal melting agarose (NMA) (prepared in MilliQ water), underside of the slide was wiped and slide was laid on flat surface to dry (First layer). To the coated slide, 75 µL 0.5% low-melting point agarose (LMPA) (prepared in PBS; $ca^{++}$, $Mg^{++}$ free) was added to prepare second layer. Third agarose layer with 80 µL of 0.5% LMPA then followed (Third layer). Slides were kept in the lysing solution (2.5M NaCl, 100

mM EDTA and 10 mM Trizma Base, 1 % Triton X-100 and 10 % DMSO were added freshly) at $4^0$ C overnight.

After lysis, slides were kept in electrophoresis chamber (Genei equipments, Banglore, India) containing electrophoresis buffer (30 ml 10N NaOH, 5 ml 200mM EDTA q.s. 1000 ml, pH>13). Slides were allowed to sit in alkaline buffer for 20 mins to allow unwinding of DNA and the expression of alkali labile damage, and then electrophoresed for 30 mins (24 volts, 300 milliamperes). Slides were then coated with the neutralization buffer (0.4 M Tris in $dH_2O$, pH 7.5).

Each cell had the appearance of a comet, with a brightly fluorescent head and a tail to one side formed by the DNA containing strand breaks that were drawn away during electrophoresis. Numbers of comet parameters were calculated with TriTek CometScore™ Freeware version 1.5 (free comet scoring software). Samples were run in duplicate, and 50 cells were randomly analyzed per slide for a total of 100 cells per sample. For qualitative analysis, cells were viewed for extent of damage and graded as shown in fig 1. For quantitative evaluation, only C0 and C1 cells were taken into account. Individual cell analysis was divided into 3 main parts. It has been documented that any change in the level of DNA damage reflect most accurately in these three parameters i.e. tail length, % tail DNA and olive tail moment[6].

In order to establish the relationship between thyroid dysfunctioning, T2DM and cellular damage, thyroid peroxidase antibody (TPO) and HbA1c in study population was measured. These were further correlated with OTM. BMI, being the major contributing factor to metabolic disorders, effect of BMI on OTM was also studied.

### Statistics

The results are expressed as mean $\pm$ S.E.M. Differences between diseased (diabetes and/or hypothyroidism) and control subjects were assessed using One-way ANOVA followed by Tukey's multiple comparison test. Correlations were performed using multiple linear regression analysis. $P<0.05$ was considered as statistically significant. All statistical calculations were performed using Microsoft excel 2007 and SPSS version 15 for Windows.

## RESULTS

### Qualitative analysis of cellular damage

The number of cells >C1 were found increased in diabetic subjects and the subjects having both diabetes and hypothyroidism (HT+DM) complications. Although, HT subjects did not show any significant cellular damage as compared to control group at $p<0.05$ (Table 1).

### Quantitative analysis of cellular damage

The whole-cell comet assay results obtained in this current study are summarized in table 2. Results of three different parameters namely, tail length (TL), % DNA in tail (TD) and olive tail moment (OTM) have been shown below.

(i) **Tail length (TL):** Tail length is the distance of DNA migration from the body of the nuclear core, which is related directly to the fragment size and it is expected to be proportional to the extent of DNA damage. As shown in table 2, DM-OHA, DM-I and HT+DM subjects demonstrated increase in tail length values (13.77 $\pm$ 0.61; 9.78 $\pm$ 0.11; 11.53 $\pm$ 0.27) ($p<0.05$) when compared with the control group (4.72 $\pm$ 0.15).

(ii) **% DNA in tail (TD):** It gives an idea regarding the damaged DNA content in individual cells, measured as the total intensity of ethidium bromide in each comet tail, verified by DNA leached out of the cell when exposed to alkaline electrophoretic conditions. It is defined as the 'ratio of tail optical intensity to the sum of tail and head optical intensity', multiplied by 100. % DNA in tail increased significantly in diabetic subjects receiving OHA (8.64 $\pm$ 0.62) and insulin therapy (7.45 $\pm$ 0.47) when compared with the control group of subjects (3.57 $\pm$ 0.16). However, there was no remarkable change observed in hypothyroid subjects (4.73 $\pm$ 0.20). Paradoxically, % DNA leached out of cell in all HT+DM (10.24 $\pm$ 0.18) subjects was found parallel with that of DM-OHA group of subjects (8.64 $\pm$ 0.62).

(iii) **Olive tail moment (OTM):** It is defined as the fraction of tail DNA multiplied by the distance between the profile centers of gravity for DNA in head and tail. OTM incorporates a measure of both the smallest detectable size of migrating DNA (reflected in the comet tail length) and the number of relaxed/broken pieces (represented by the intensity of DNA in the tail). OTM parameter was influenced in the similar pattern to that of tail DNA parameter. OTM increased significantly in DM-OHA (0.89 $\pm$ 0.06), DM-I (0.68 $\pm$ 0.03) and HT+DM subjects (0.58 $\pm$ 0.02) when compared with that of control group (0.18 $\pm$ 0.008).

Finally, the individual cell analysis in terms of tail length, % DNA in tail and olive tail moment was intercorrelated using multiple linear regression analysis. As can be seen from table 4, OTM was found best correlated with tail length in DM-OHA (R= 0.92), DM-I (R= 0.92), HT (0.77) and HT+DM (R= 0.77) group of subjects. Further, OTM was also found well correlated with % DNA in tail in all groups viz., control (R= 0.71), DM-OHA (R= 0.95), DM-I (R= 0.94), HT (R= 0.86) and HT+DM (R= 0.81) subjects. Thus, OTM appeared to be the most statistically significant measurement.

### Effect of TPO antibody, HbA1c and BMI on OTM

TPO, a typical marker of thyroid dysfunctioning was elected to evaluate the impact of hypothyroidism on cell damage in terms of OTM. The correlation between these two parameters could not be established by the current study (figure 3). i.e. change in TPO levels did not directly make an indication of the change in tail length.

HbA1c, a clinical marker of diabetes mellitus signify the blood sugar control for previous three months records. As per our study, diabetic subjects showed statistical significant HbA1c values as compared to control. Therefore, an effort was made to match the correlation between HbA1c and OTM. The correlation coefficient ($R^2$) value was 0.98 (figure 4) indicating significant positive correlation between HbA1c and OTM.

BMI, a major predictor of obesity and oxidative stress may be a contributor of cell damage. Both diabetes mellitus and hypothyroidism are two major metabolic disorders highly prevalent in obese individuals. The correlation between BMI and OTM reflected a significant association in our study at $R^2$=0.91 (Linear regression) (figure 5). Persons with BMI values <25 kg/m$^2$, showed OTM of 0.42 (arbitrary units) and as the value of BMI increased to 40 or more, the corresponding OTM reached to 0.65.

### Effect of drug treatments on Tail length

All the subjects were grouped according to their pharmacological treatment for corresponding metabolic disorder(s). As shown in fig 6, 'L' receiving subjects had lower values for OTM. Contrary to this, subjects receiving 'S' showed significantly high OTM values. Further, subjects receiving both 'S+L' therapy showed significant OTM measurements. It is to be noted that subjects receiving 'S' either alone or in combination with insulin or metformin also reflected higher ($p<0.05$) OTM values.

## DISCUSSION

In the current study, we attempted to define the most reliable comet measurements that would truly reflect the extent of DNA damage induced by metabolic disorders namely, type 2 diabetes mellitus (T2DM) and hypothyroidism. We approached this methodology by performing the alkaline comet assay on peripheral blood samples of subjects with any one or both of the metabolic disorders. For qualitative evaluation, we measured the number of highly damage cells (>C1). In our study, we found that percentage of "ghost cells" (>C1) was significantly high in numbers in diabetic subjects alone or in combination with hypothyroid complications. These results show that presence of diabetes may contribute towards early cell damage of these subjects. In the comet assay, the results of quantitative evaluation clearly show an increase in comet parameters tail length, % DNA in tail and olive tail moment, calculated by taking into account C0 and C1 cells.

**Table -1: Qualitative evaluation of cell damage in metabolic disorder(s)**

| | Control | HT | DM-OHA | DM-I | HT+DM |
|---|---|---|---|---|---|
| **C0** | 73.50 ± 2.56 | 43.20 ± 1.58 | 7.23 ± 0.24* | 23.40 ± 1.45 | 3.97 ± 0.65* |
| **C1** | 25.40 ± 1.25 | 54.93 ± 3.14 | 67.20 ± 3.88* | 77.17 ± 3.14* | 79.15 ± 3.25* |
| **>C1** | 1.10 ± 0.02 | 1.87 ± 0.05 | 15.60 ± 1.04* | 9.40 ± 0.63* | 16.88 ± 0.90* |

All the data are shown as mean ± S.E.M. values. *, p< 0.05: statistically significant difference from controls. HT= hypothyroid subjects, DM- OHA= diabetic subjects receiving oral hypoglycemic agents, DM-I= diabetic subjects receiving Insulin, HT+DM= subjects receiving treatment for both diabetes and hypothyroidism

**Table 2. Impact of various metabolic disorder(s) on comet assay indices**

| Parameter | Control | DM-OHA | DM-I | HT | HT+DM |
|---|---|---|---|---|---|
| **Comet Length (μm)** | 66.11 ± 1.652 | 125.67 ± 1.77* | 106.54 ± 1.29* | 70.41 ± 0.77 | 73.55 ± 1.66 |
| **Comet Height (μm)** | 71.35 ± 1.69 | 118.22 ± 1.56* | 107.52 ± 1.17* | 77.36 ± 1.39 | 81.17 ± 1.82 |
| **Comet Area ($\mu m^2$)** | 5028.63 ± 252.69 | 13685.34 ± 282.82* | 9456.90 ± 245.09* | 5723.62 ± 203.73 | 6202.35 ± 375.02 |
| **Comet Intensity** | 37970.62 ± 3105.76 | 87998.48 ± 2910.99* | 51724.39 ± 2291. 60 | 32773.37 ± 3023.80 | 49249.37 ± 4043.17 |
| **Comet Mean Intensity** | 66.73 ± 3.08 | 59.70 ± 1.00 | 55.12 ± 0.58 | 49.12 ± 3.85* | 75.39 ± 1.52 |
| **Head Diameter (μm)** | 63.37 ± 1.80 | 112.44 ± 1.78* | 96.88 ± 1.27* | 63.19 ± 1.53 | 62.85 ± 1.49 |
| **Head Area ($\mu m^2$)** | 4997.54 ± 252.76 | 13180.68 ± 285.12* | 8903.74 ± 239.20* | 5623.65 ± 206.01 | 5988.27 ± 374.22 |
| **Head Intensity** | 37036.08 ± 3061.86 | 83118.41 ± 2791.70* | 48110.47 ± 2164.53* | 31229.97 ± 2974.79 | 46234.14 ± 3850.99 |
| **Head Mean Intensity** | 65.15 ± 3.00 | 57.71 ± 1.01* | 54.21 ± 0.49* | 47.61 ± 3.74 | 70.80 ± 1.41 |
| **% DNA in Head** | 96.44 ± 0.16 | 91.36 ± 0.62* | 92.55 ± 0.47* | 95.27 ± 0.20 | 89.77 ± 0.18* |
| **Tail Length (μm)** | 4.72 ± 0.15 | 13.77 ± 0.61* | 9.78 ± 0.11* | 8.78 ± 0.39 | 11.53 ± 0.27* |
| **Tail Area ($\mu m^2$)** | 31.08 ± 1.22 | 504.66 ± 34.43* | 553.16 ± 17.69* | 99.97 ± 9.67 | 214.08 ± 6.58* |
| **Tail Intensity** | 934.54 ± 78.07 | 4880.07 ± 199.28* | 3613.92 ± 200.70* | 1543.40 ± 38.84 | 3015.23 ± 264.62* |
| **Tail Mean Intensity** | 514.75 ± 69.39 | 1261.18 ± 220.69 | 1158.21 ± 32.24 | 360.97 ± 85.15 | 708.19 ± 36.21 |
| **%DNA in Tail** | 3.57 ± 0.16 | 8.64 ± 0.62* | 7.45 ± 0.47* | 4.73 ± 0.20 | 10.24 ± 0.18* |
| **Tail Moment** | 0.075 ± 0.003 | 0.62 ± 0.06* | 0.43 ± 0.05* | 0.24 ± 0.02 | 0.55 ± 0.04* |
| **Olive Tail Moment** | 0.18 ± 0.008 | 0.89 ± 0.06* | 0.68 ± 0.03* | 0.32 ± 0.01 | 0.58 ± 0.02* |

All the data are shown as mean ± S.E.M. values. *, p< 0.05: statistically significant difference from controls . HT= hypothyroid subjects, DM-OHA= diabetic subjects receiving oral hypoglycemic agents, DM-I= diabetic subjects receiving Insulin, HT+DM= subjects receiving treatment for both diabetes and hypothyroidism

**Table 3. Comparison of some commonly used metrics in leucocytes of hypothyroid and/or diabetic subject with control**

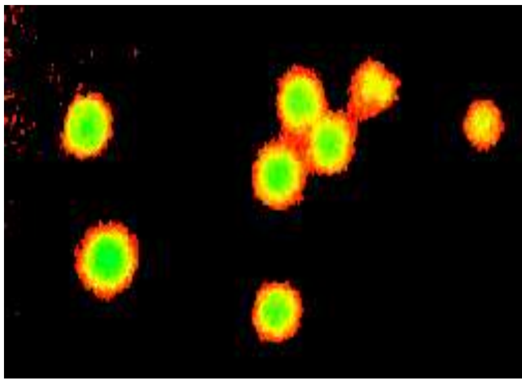
| | DM-OHA | DM-I | HT | HT+DM |
|---|---|---|---|---|
| **Tail length** | | | | |
| Mean difference | -9.056* | -5.057* | -4.057 | -6.809* |
| C.I. of mean | 1.385 | 0.258 | 0.873 | 0.605 |
| **Tail DNA** | | | | |
| Mean difference | -5.073* | -3.882* | -1.164 | -6.670* |
| C.I. of mean | 1.391 | 1.051 | 0.439 | 0.396 |
| **Olive tail moment** | | | | |
| Mean difference | -0.710 | -0.504 | -0.146 | -0.402 |
| C.I. of mean | 0.130 | 0.066 | 0.024 | 0.056 |

Mean difference represents the difference with control values. Least significant difference was evaluated and confidence interval of mean values was calculated. *, p< 0.05: statistically significant difference from controls.

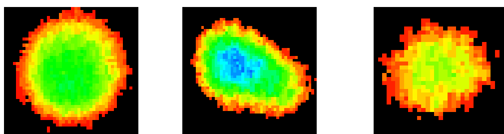
**Table 4. Multiple linear regression analysis between tail length, % DNA in tail and olive tail moment**

| Parameters | | Control | DM-OHA | DM-I | HT | HT+DM |
|---|---|---|---|---|---|---|
| TL and TD | R | -0.351 | 0.802** | 0.802** | 0.615* | 0.615* |
| | p | 0.321 | 0.005 | 0.005 | 0.044 | 0.044 |
| TL and OTM | R | -0.305 | 0.918*** | 0.918*** | 0.772** | 0.772** |
| | p | 0.392 | <0.001 | <0.001 | 0.005 | 0.005 |
| TD and OTM | R | 0.71* | 0.947*** | 0.941*** | 0.855*** | 0.814** |
| | p | 0.0215 | <0.001 | <0.001 | <0.001 | 0.004 |

Values are shown as Pearson correlation coefficient (R) followed by pvalues. Correlation coefficient '0' indicates no correlation and '1' indicates 100 % correlation. *, p< 0.05: statistically significant from each other**, p< 0.01: statistically significant from each other ***, p< 0.001: statistically significant from each other TL= tail length, TD= % DNA in tail, OTM= olive tail moment



(a)



(b)

**Figure -1:** Comet assay image. (a) Ethidium bromide stained slide when viewed under fluorescent microscope at 20x. (b) Comet notation. Cells noted 0 (C0): undamaged cells; Cells noted 1 (C1): mild and moderate damaged cells; C>1: high damage and “ghost” cells

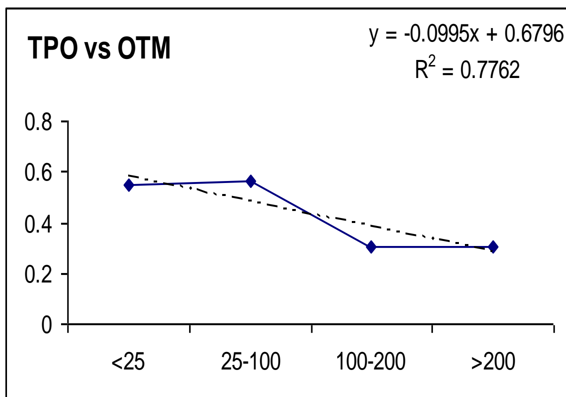



**Figure -3:** Correlation between TPO and OTM (Values of OTM are depicted against different levels of TPO antibodies)

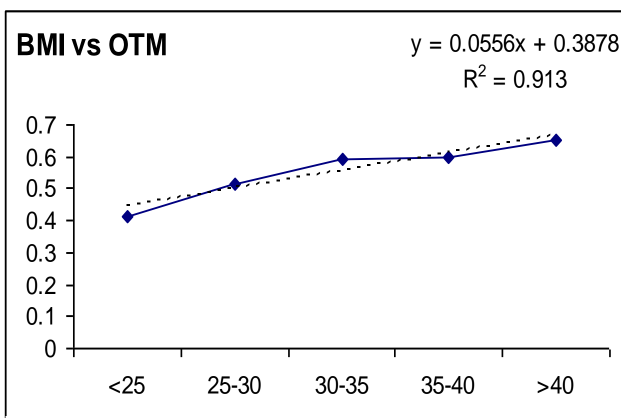



**Figure -5:** Correlation between BMI and OTM (Values of OTM are depicted against different ranges of BMI values. Linear regression analysis showed $R^2$=0.913 and equation y= 0.39x + 0.45)

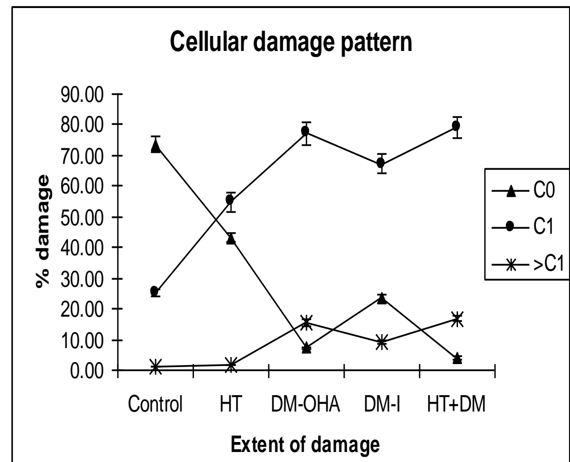



**Figure -2:** Cellular damage pattern (All the data are shown in mean ± S.E.M. values. C0= undamaged cells, C1= mild and moderate damage cells, >C1= high damage and ghost cells. HT= hypothyroid subjects, DM-OHA= diabetic subjects receiving oral hypoglycemic agents, DM-I= diabetic subjects receiving Insulin, HT+DM= subjects receiving treatment for both diabetes and hypothyroidism)

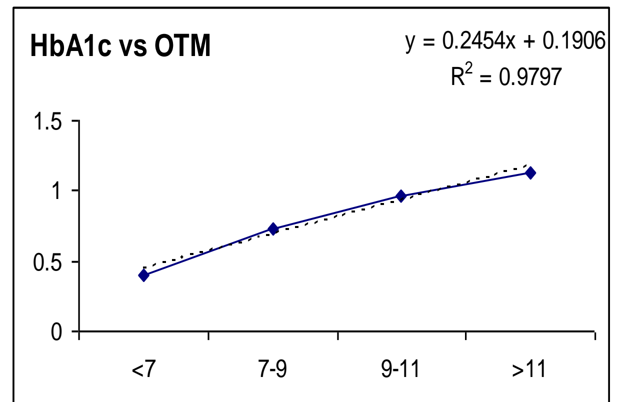



**Figure -4:** Correlation between HbA1c and OTM (Values of OTM are depicted against different levels of HbA1c. Linear regression analysis showed $R^2$=0.966 and equation y= 0.39x + 0.45)

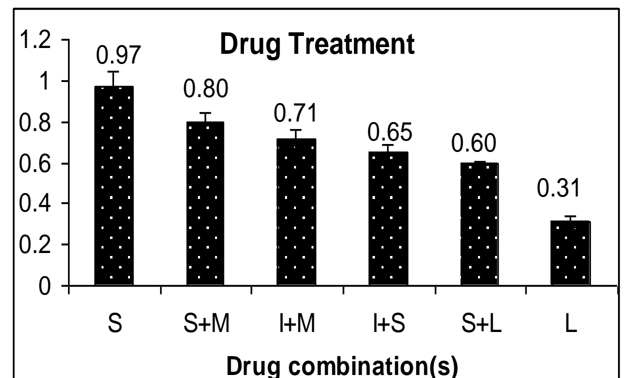



**Figure -6:** Comparison between various drug treatments (All the data are shown as mean ± SEM. Values of OTM are compared against control.Significantly different when compared with the control at $p<0.05$. L=levothyroxine, I+M= insulin and metformin in combination, S+L= sulphonamide and levothyroxine in combination, I+S= insulin and sulphonamide in combination, S+M= sulphonamide and metformin in combination, S= sulphonamide drug treatment)

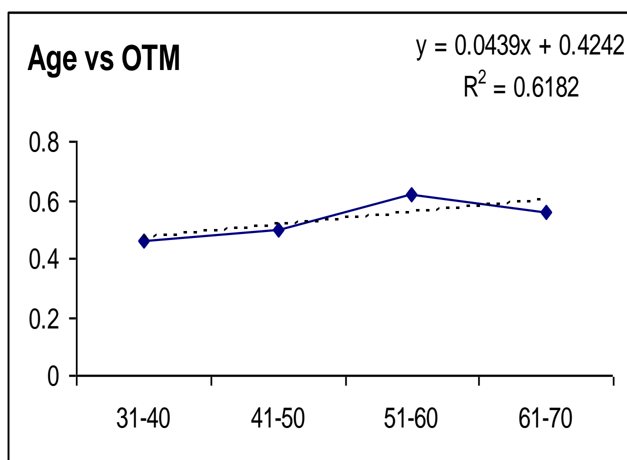



**Figure -7:** Age wise distribution of OTM (Values of OTM are depicted against different ranges of age. Linear regression analysis showed $R^2$=0.618 and equation y= 0.0439x + 0.4242)

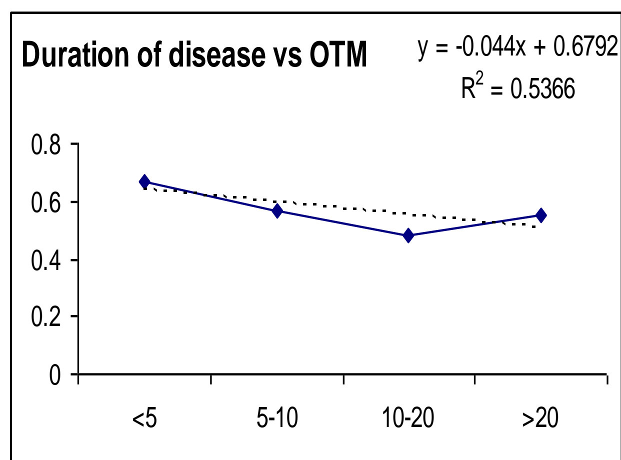



**Figure -8:** Duration of disease condition and distribution of OTM (Values of OTM are depicted against different ranges of age. Linear regression analysis showed $R^2$=0.537 and equation y= -0.044x + 0.6792)

**DISCUSSION**

In the current study, we attempted to define the most reliable comet measurements that would truly reflect the extent of DNA damage induced by metabolic disorders namely, type 2 diabetes mellitus (T2DM) and hypothyroidism. We approached this methodology by performing the alkaline comet assay on peripheral blood samples of subjects with any one or both of the metabolic disorders. For qualitative evaluation, we measured the number of highly damage cells (>C1). In our study, we found that percentage of "ghost cells" (>C1) was significantly high in numbers in diabetic subjects alone or in combination with hypothyroid complications. These results show that presence of diabetes may contribute towards early cell damage of these subjects. In the comet assay, the results of quantitative evaluation clearly show an increase in comet parameters tail length, % DNA in tail and olive tail moment, calculated by taking into account C0 and C1 cells.

Quantitative analysis of comet assay allows detection of very low levels of DNA strand breaks induced by oxidative stress in metabolic disorders. Broadly, there are two types of comet assay measurements, 'primary' and 'derived'. Primary measurements are obtained from primary densitometric analysis of the comet images (DNA profile across the comet). These are (a) Length of DNA migration (b) Percentage of DNA in tail (c) Distribution profile of DNA in the tail.

The length of DNA migration, commonly referred to as the tail length (if measured from the centre of the head to the end of the tail) is the first comet measurement outcome used to quantify DNA damage. The length of DNA migration is directly related to the loops of released DNA or the size of DNA fragments, and is proportional to the amounts of strand breaks and alkali-labile sites[6]. DM-OHA, DM-I and HT+DM subjects in our study also showed significantly raised values of tail length when compared with the control group ($p<0.05$). The 'percentage DNA in the tail' is the second primary comet measurement on which other derived units are based. The percentage of DNA in tail is directly proportional to the amount of damaged DNA[7]. It was extremely significant in DM-OHA, DM-I and HT+DM subjects as compared to other groups.

Our results are in agreement with the study by Dandona et al., who reported a high concentration of 8-oxo-dGuo (typical product of DNA oxidation) released into blood after excised from DNA by repair enzyme in the lymphocytes of type-1 diabetes mellitus (T1DM) and T2DM[8]. High blood glucose levels in vitro may impair cellular DNA repair and increase DNA cleavage[9]. Diabetes and hyperglycemia can lead to DNA damage via the oxidation of DNA bases and sugar-phosphate binding sites[10]. Our previous clinical findings of increased serum levels of lipids, differences in creatinine levels, glycated haemoglobin levels and microalbuminuria (submitted for publication) in all the patients suffering from T2DM supports the hypothesis that diabetes mellitus disorder related changes in the body might contribute to DNA damage or cell death.

As can be seen from our results, hypothyroid subjects did not show any change in tail length, % DNA in tail and olive moment ($p>0.05$) when compared with the control group. It has been proposed that the basal metabolic rate reduced by hypothyroidism has two major effects on the intracellular redox environment and cellular damage. One is its protective mechanism against toxin producing oxidative stress[11,12] and the other is mechanism of hypothyroidism-induced oxidative stress and cellular damage[13,14]. Our results support the first hypothesis, and the protective effect may be attributed to the action of thyroid hormones on regulation of cell cycle.

Any changes in the level of DNA damage will be reflected most accurately by OTM measurement6. OTM is well correlated with the tail length and tail DNA in different groups. Further, presence of raised anti-TPO antibodies has been known to indicate subclinical or clinical presence of thyroid disease15. To understand the effect of TPO antibody on cell damage, the OTM parameter was studied using different ranges of TPO antibody (<25 to >200 IU/l). No significant correlation could be established with TPO and tail length by our study. It may again suggest the hypothesis that changes in thyroid hormone levels do not significantly affect the cell damage.

HbA1c predicts control of blood sugar levels in diabetic individuals. Further, role of hyperglycemia and obesity are established as an important risk factors for DNA damage[9,16]. Based on our results of the $R^2$ value of HbA1c and BMI we support above hypothesis. Further, Kaneto et al. have reported that hyperglycemia itself contributes to increased generation of ROS and increased oxidative stress leading to oxidative DNA damage[9]. BMI, has been indicated as an important risk factor for various metabolic disorders[16]. Our observations have also shown correlation (Linear regression) between increase in BMI with that of OTM values at $R^2$ value of 0.91 (Fig 5).

Certain drugs are known to induce DNA damage in healthy cells and potentiate the oxidative stress generated during cellular events. Induction of DNA damage in lymphocytes by various antibiotics[17], anaesthetic agents[18] and oral contraceptives[19] have been studied with the help of comet assay. To the best of our knowledge, no study has been performed to understand the effect of anti-diabetic drugs, and thyroid hormone replacement therapy on cell damage with the help of comet assay. We have also observed that subjects receiving sulphonamide therapy showed significantly higher OTM measurements revealing greater possibility of cell damage. Thus, in the light of our observation, it is suggested that diabetes patients showed increased DNA damage as significant differences were detected between control, hypothyroid and diabetic patients in terms of frequencies of damaged cells.

## CONCLUSION

In conclusion, we suggest the presence of significant cellular damage in diabetic population and diabetic hypothyroid complications as compared to hypothyroid patients and normal healthy individuals, which is suggestive of detrimental effects of diabetes. Further, the DNA damaging effects of drug treatment like sulphonamides can not be ruled out as patients receiving glibenclamide showed high OTM measurements indicating greater possibility of cellular damage. The high level of concordance of the results obtained in the comet assay showed that the comet assay is not only sensitive enough to detect low levels of DNA damage in human lymphocytes, but it is also highly specific and give an idea of diabetes control in T2DM individuals.

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