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LIPID PEROXIDATION: A BIOMARKER OF OXIDATIVE STRESS IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

Type 2 Diabetes Mellitus (T2DM) is a chronic disease characterized by hyperglycemia with serious metabolic disturbances in carbohydrate, protein and fat metabolism due to impaired insulin deficiency or insulin action and associated with increased free- radical activity. An increased free radical production has been observed in diabetic patients leading to increased oxidative stress. Oxidative stress (OS) represents a redox imbalance between the production and manifestation of reactive oxygen species (ROS) especially free radicals and a biological system's ability i.e. the endogenous antioxidant systems to detoxify the reactive intermediates or to repair the resulting damage. Free oxygen radicals may lead to DNA mutations, changes in structure and function of proteins, and peroxidation of lipids in cell membrane by forming reactions with macromolecules. Lipid peroxidation, owing to free-radical activity, plays an important role in the development of complications of T2DM. The purpose of the study was designed to evaluate plasma malondialdehyde (MDA) levels measured as thiobarbituric acid-reactive substances (TBARS) (index of lipid peroxidation) in T2DM patients compared to the healthy normal subjects in population of West Bengal. Levels of MDA were significantly higher in diabetic samples rather than the healthy controls. Elevated levels of MDA may be a useful biomarker of OS. Enhanced lipid peroxidation leads to an increase in free radical activity in T2DM. Increase in free radical activity in T2DM along with insulin resistance leads to activation of stress-sensitive intracellular signaling pathways which may play an important role in complications of T2DM.

Keywords: Type 2 diabetes mellitus, Oxidative stress, Lipid peroxidation, Malondialdehyde

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a chronic disease characterized by hyperglycemia with serious metabolic disturbances in carbohydrate, protein and fat metabolism due to impaired insulin deficiency or insulin action and associated with increased free-radical activity. The number of people with T2DM is increasing in every country with 80% of people with DM living in low- and middle-income countries and has affected approximately 347 million people (Brussels, 2011); WHO. T2DM is responsible for alteration in lipid profile, especially an increased susceptibility to lipid peroxidation (Giugliano *et al.*, 1996). An increased free radical production has been observed in diabetic patients leading to increased oxidative stress (Seghrouchni *et al.*, 2002). The mechanisms of free-radical production include advanced glycated end products formation, protein glycation, glucose autoxidation and activation of polyol pathway, ultimately resulting in oxidative stress in a variety of tissues (Atalay *et al.*, 2002). Oxidative stress (OS) represents a redox imbalance between the production and manifestation of reactive oxygen species (ROS) especially free radicals and a biological system's ability i.e. the endogenous antioxidant systems to detoxify the reactive intermediates or to repair the resulting damage leading to the activation of stress-sensitive intracellular signaling pathways (Evans *et al.*, 2002). Hence, oxidative stress may be implicated in the pathogenesis of diabetes.

The increased production of reactive oxygen species can stimulate chain reactions by interacting with proteins, lipids, and nucleic acids causing cellular dysfunction. Increased free radical production mediates tissue injury

in a wide range of diseases including T2DM (Yildiz *et al.*, 2002). Free oxygen radicals may lead to DNA mutations, changes in structure and function of proteins, and peroxidation of lipids in cell membrane by forming reactions with macromolecules. Lipid peroxidation, owing to free-radical activity, plays an important role in the development of complications of diabetes. T2DM causes oxidative degradation of lipids in cell membranes resulting in cell damage. Lipid degradation leads to increased susceptibility to lipid peroxidation. Excessively high levels of free radicals cause damage to cellular organelles and enzymes, increases lipid peroxidation, and development of insulin resistance that leads to diabetic complications. ROS degrade polyunsaturated lipids present on cell membrane forming stable aldehydes such as malondialdehyde that will damage the cell membranes. This aldehyde product is used as a biomarker to estimate the level of OS in an organism as it is a marker for OS. Although increased levels of lipid peroxidation, as a consequence of free radical activity, have been reported in both type 1 and type 2 diabetes with vascular complications (Griesmacher *et al.*, 1995; Jennings PE *et al.*, 1991). Many studies failed to indicate any significant elevation in lipid peroxidation in diabetic patients, probably owing to heterogeneity of the patient population (Velazquez *et al.*, 1991). It is worth mentioning that the authors themselves highlighted the need to conduct further studies to validate this investigation.

Thus the present study was designed to evaluate lipid peroxidation end product such as plasma malondialdehyde (MDA) levels measured as thiobarbituric acid-reactive substances (TBARS) (index of lipid peroxidation) in T2DM patients compared to the healthy normal subjects in population of West Bengal.

MATERIALS & METHODS

Study setting and subjects

The study was conducted at Vivekananda Institute of Medical Sciences, Kolkata,West Bengal. The study was held on successive patients after obtaining informed consent approved by the Ethical Clearance Committee of the Institution. A total of one hundred and seventeen patients (fifty four male patients and sixty three female patients) with T2DM and thirty two healthy control subjects (twelve male controls and twenty female controls) were recruited from different areas of West Bengal. The patients were confirmed of having T2DM by impaired fasting glucose test (>126 mg/dl) and oral glucose tolerance test (>200 mg/dl). The controls had a self reported history of having normal glucose metabolism. Detailed personal histories were collected from the participants with the help of questionnaire including age, height, weight, bmi, address, family history of T2DM, duration for having T2DM, complications of T2DM etc. We had excluded the patients who had fever, acute and chronic infections, malignancy, acute and chronic nephritis, cirrhosis, and congestive heart failure, smokers, patients having other hypoglycemic drugs or other antioxidant therapy. All the patients were under stable conditions during assessment.

Collection of peripheral blood

Peripheral blood samples were collected by venipuncture both from T2DM patients and healthy control subjects. Plasma was separated by centrifugation at 2500rpm for 20 mins. Plasma was then futher analysed for MDA level estimation. MDA was measured spectrophotometrically by the method of Satoh (1978).

Estimation of plasma malondialdehyde (Satoh, 1978)

Plasma malondialdehyde estimated by Kei Satoh method. It is based on the principle of auto-oxidation of unsaturated fatty acids involving the formation of semistable peroxides, which then undergo a couple of reactions to form malondialdehyde (MDA). MDA reacts with thiobarbituric acid (TBA) to form pink colored chromogen. The resulting chromogen is extracted with 4.0ml of n-butyl alcohol and the absorbance of which is measured at 530 nm.

Statistical Analysis

All variables were expressed as mean ± SD (standard deviation). Mean values obtained from sample and control groups were compared by Independent t-test. A value of P <0.5 was considered as statistically significant. All statistical analyses were performed by using statistical software PASW Statistical Viewer.

RESULTS

The clinical characteristics of the study participants are listed in Table 1. The study participants included both

men and women and the clinical characteristics incorporated personal history of education, marital status, smoking, antioxidant supplement consumption, or the practice of physical activity. All the study participants were from Bengalee culture.The mean age of the female T2DM patients was 51.89 ± 12.46 years ranging between 30 - 85 years, with a mean duration of the diabetes of 7.2 ± 5.8 years (range: 1–22 years). Whereas, the mean age of the male T2DM patients was 54.1 ± 10.8 years ranging between 30 – 85 years, with mean duration of diabetes of 8.1 ± 7.6 years (range: 1–34 years). Diabetic individuals had higher BMI (body mass index) than the healthy controls. Impaired fasting glucose, Oral glucose tolerance test and HbA1C were significantly higher in T2DM patients than the control group participants.

Table I: General characteristics of the study participants

Criteria		**Groups**			
		Controls (n= 32)		T2DM patients (n= 117)	
Gender	Male	12(37.5%)		54(46.2%)	
	Female	20(62.5%)		63(53.8%)	
		Male	Female	Male	Female
Age (years)		46.58± 17.9	41.85± 13.2	50.4±18.1	51.89±12.46
Duration of diabetes (years)				8.1±7.6	7.2±5.8
BMI(kg/m^2)		24.4± 2.5	23.9±2.26	24.11±2.53	23.93±3.82
Smoking		2(16%)	_____	22(41%)	_____
Marital Status	Married	10 (83.3%)	20(100%)	53(98.1%)	59(93.65%)
	Unmarried	2 (16.7%)	_____	1(1.85%)	1(1.59%)
	Widow	_____	_____	_____	3(4.76%)
Physical Activity		2(16%)	_____	9(16%)	8(13%)
Education (no. of cases)	None	1(8.3%)	10(50%)	4(7.4%)	21(33.3%)
	Elementary	8(66.7%)	8(40%)	33(61.1%)	33(52.4%)
	Bachelor	3(25%)	2(10%)	17(31.5%)	9(14.3%)
Antioxidant Supplementation		6(5%)	4(20%)	1(2%)	5(8%)

[N.B: (Mean ± SD; unless otherwise specified); BMI: body mass index.]

Socioeconomic status (SES), particularly income and educational qualification, are the two most important factors responsible for higher prevalence of T2DM, regardless of various sociodemographic factors that may contradict or mediate these associations. Prevalance of T2DM may be high in low income populations in addition with poor educational qualification (Robbins *et al*., 2001; National Public Health Survey., 1998; Stelmach *et al*., 2005). Present study on SES and the prevalance of diabetes suggested that higher educational qualification was associated with a lower risk of T2DM Lee (TC *et al.*, 2011). Poor educational qualification in addition with lower income has been considered as a predictor affecting poor health outcomes, management of chronic disease and crisis in services of monetory value (Choi AI *et al.*, 2011; Adler *et al.*, 1994). Our present study reveals that most of the patients had elementary education and a pattern of higher prevalence towards the lowest and medium household income after adjustment for various sociodemographic factors, depicting that income and educational attainment are cognitive factors of T2DM among population of West Bengal, presented in figure I and II. Previous studies stated that income level, which was a major reflection of SES, was closely associated with the adverse health effects including the incidence of T2DM across studies and across cultures (Rabi *et al.*, 2006; Krishnan *et al.*, 2010; Lysy *et al.*, 2013; Espelt *et al.*, 2008). For instance, individuals with a lower SES were at higher risk of T2DM (Raphael *et al.*, 2003; Dinca-Panaitescu *et al*., 2011). Hence, our findings also support the association between SES and the prevalence of T2DM, implying that higher income is an indicator of having better access to goods and service of greater monetary value that leads to an affordable and healthier lifestyle.

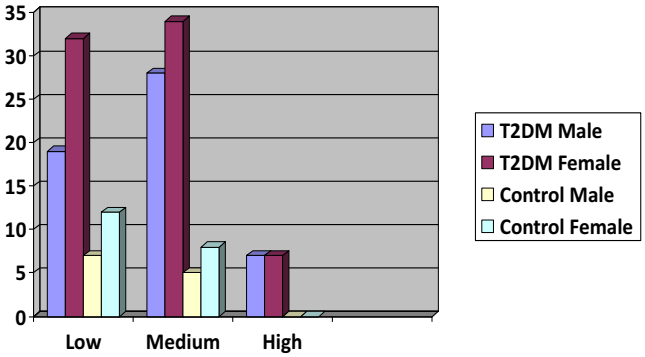

Figure I: Socioeconomic status of control group participants and T2DM[N.B: Low- Upto Rs5000/- per month, Medium - Above Rs.5000/- to below Rs. 20000/- per month, Good- Above Rs. 20000/- per month.]

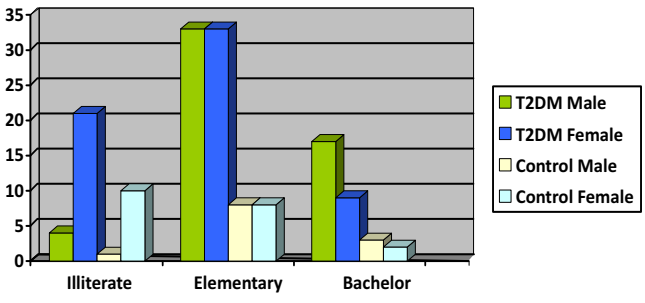

Figure II: Educational Qualification of control group participants and T2DM

Table II: Lipid Peroxidation in Studied Participants

Type of Individuals	Lipid Peroxidation (nM/ml) [Mean ± SD]
Healthy Control	3.32 ± 1.56
T2DM without complications	4.89 ± 2.67
T2DM with Macrovascular complications	4.88 ± 4.47
T2DM with Microvascular complications	5.02 ± 2.31

Plasma MDA level was compared based on diabetic complications. Patients were divided on three groups depending on their complications. T2DM complications included macrovascular (due to damage to larger blood vessels) and microvascular (due to damage to small blood vessels) complications. Thus the following groups were T2DM without complications, T2DM with macrovascular complications and T2DM with microvascular complications. Macrovascular complications include ischemic heart disease, peripheral vascular disease, and cerebrovascular disease. Whereas microvascular complications include retinopathy, nephropathy and neuropathy.In the present study, significantly elevated plasma MDA level ($p \leq 0.05$) was found in T2DM patients (4.81 ± 3.27 nM/ml) compared to healthy control subjects (3.42 ± 1.65nM/ml). Among three groups of T2DM, MDA level was found statistically significantly higher among T2DM patients with microvascular complications (5.02 ± 2.31nM/ml, $p \leq 0.5$) than patients with macrovascular complications (4.88 ± 4.47 nM/ml, $p \leq 0.5$) and patients without complications (4.81 ± 2.67 nM/ml, $p \leq 0.5$) as compared to healthy controls (3.43 ± 1.65 nM/ml) respectively.

DISCUSSION

T2DM has been known to be a state of increased production of free radicals contributed by several mechanisms, including hyperglycemia and antioxidant defences leading to OS. Increased OS exacerbates the development and progression of diabetes and its complications Ceriello A., 2000; Baynes *et al.*, 1999; Baynes , 1991. Mechanisms by which increased OS is involved in the diabetic complications are partly known, including activation of transcription factors, advanced glycated end products (AGEs), and protein kinase C. Increased production of free radicals observed in T2DM and its insufficient removal results in damage to cellular proteins, membrane lipids and nucleic acids.

Elevated lipid peroxidation in the plasma and cells can arise from factors responsible for the formation of ROS. In poorly controlled T2DM, glucose oxidation through the pentose phosphate pathway leads to the excessive production of NADPH, which in turn leads to lipid peroxidation in presence of cytochrome *P*-450 system. Oxyhaemoglobin in erythrocytes may act like the cytochrome *P*-450 system in the presence of NADPH which in turn promotes to excessive lipid peroxidation (Sundaram *et al.*, 1996) .Alternatively, inhibition or inactivation of antioxidant enzymes by glycosylation in poorly controlled T2DM may give rise to increased lipid peroxidation.Previous studies have also reported evidence of lipid peroxidation in T2DM progression and its complications (Sundaram *et al.*, 1996; Zigler *et al.*, 1985). Several authors have documented increased levels of lipid peroxidation in T2DM patients (Akkus I *et al.*, 1996; Kesavulu *et al.*, 2001; Sundaram *et al.*, 1996; Nutthall *et al.*, 1999; Buyukkocak *et al.*, 2000) and others could not find a significant increase in lipid peroxidation in diabetics (Sundaram *et al.*, 1996; Zigler JS *et al.*, 1985).

In the present study, elevated plasma MDA level was found in T2DM patients compared to healthy control subjects. Among three groups of T2DM, T2DM with microvascular complications showed highest increase in MDA level compared to healthy controls. Thus the observed increase in MDA levels may be due to the enhanced production of lipid peroxides and their release in circulation that leads to increased lipid peroxidation which is responsible for T2DM progression and its complications and can be a risk factor for development of cancer (Jamuna Rani A *et al.*, 2014; Benrebai M *et al.*, 2008). Hisalkar *et al* stated that diabetic complications are developed with increased activity of free radical – induced lipid peroxidation and accumulation of lipid peroxidation products (Hisalkar *et al.,* 2012).

CONCLUSION

This investigation suggest that hyperglycaemia in T2DM leading to OS result in an increase in lipid peroxidation. Increase in lipid peroxidation reveals increased production of free radicals and its insufficient removal by antioxidants. Enhanced MDA level is a prognostic factor for the risk of T2DM progression and its complications. Hence, lipid peroxidation can be a useful biomarker for OS for the risk of T2DM progression and its complications.

Adler, N.E., Boyce, T., Chesney, M.A., Cohen, S., Folkman, S., Kahn, R.L. & Syme, S.L. (1994). Socioeconomic status and health. The challenge of the gradient. *American Psychologist*, 49(1), pp15-24.

Akkus, I., Kalak, S., Vural, H., Caglayan, O., Menekşe, E., Can, G. & Durmuş, B. (1996). Leukocyte lipid peroxidation, superoxide dismutase, glutathione peroxidase and serum and leukocyte vitamin C levels of patients with type II diabetes mellitus. *Clinica Chimica Acta*, 244(2), pp 221–227.

Atalay, M. & Laaksonen, D. E. (2002). Diabetes, Oxidative stress and physical exercise. *Journal of Sports Science and Medici*ne, 1(1), pp 1-14.

Baynes, J.W. & Thorpe, S.R. (1999). Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. *Diabetes*, 48(1), pp 1–9.

Baynes, J.W. (1991). Role of oxidative stress in development of complications in diabetes. *Diabetes*, 40(4), pp 405–412.

Benrebai, M., Abidli, N., Nasr, S.M. & Benlatreche, C. (2008). Oxidative stress status in type 2 diabetic patients in Eastern Algeria. *World Applied Sciences Journal*, 4(5), pp 714–719

Brussels. 2011. Global burden of diabetes. International Diabetes federation, in: Diabetic atlas. 5th edition. Retrieved from: http://www.idf.org/diabetesatlas.

Buyukkocak, S., Ozturk, H.S., Tamer, M.N., Kacmaz, M., Cimen, M.Y. & Durak, I. (2000). Erythrocyte oxidant/antioxidant status of diabetic patients. *Journal of Endocrinological Investigation*, 23(4), pp 228–230.

Ceriello, A. (2000). Oxidative stress and glycemic regulation. *Metabolism*, 49(2), pp 27–29.

Choi, A.I., Weekley, C.C., Chen, S-C., Li, S., Tamura, M.K., Norris, K.C. & Shlipak, M.G. (2011). Association of educational attainment with chronic disease and mortality: the Kidney Early Evaluation Program (KEEP). *American Journal of Kidney Diseases*, 58(2), pp 228–34.

Dinca-Panaitescu, S., Dinca-Panaitescu, M., Bryant, T., Daiski, I., Pilkington, B. & Raphael, D. (2011). Diabetes prevalence and income: results of the Canadian Community Health Survey. *Health Policy*, 99(2), pp116–23.

Espelt, A., Borrell, C., Roskam, A.J., Rodríguez-Sanz, M., Stirbu, I., Dalmau-Bueno, A., Regidor, E., Bopp, M., Martikainen, P., Leinsalu, M., Artnik, B., Rychtarikova, J., Kalediene, R., Dzurova, D., Mackenbach, J. & Kunst, A.E. (2008). Socioeconomic inequalities in diabetes mellitus across Europe at the beginning of the 21st century. *Diabetologia,* 51(11), pp1971-1979.

Evans, J.L., Goldfine, I.D., Maddux, B.A. & Grodsky, G.M. (2002). Oxidative stress and stress-activated signaling pathways: A unifying hypothesis of type 2 diabetes. *Endocrine Reviews*, 23(5), pp 599-622.

Giugliano, D., Ceriello, A. & Paolisso, G. (1996). Oxidative stress and diabetic vascular complications. *Diabetes Care,* 19(3), 257-267.

Griesmacher, A., Kindhauser, M., Andert, S.E., Schreiner, W., Toma, C., Knoebl, P., Pietschmann, P., Prager, R., Schnack, C. & Schernthaner, G. & Mueller, M.M. (1995). Enhanced serum levels of thiobarbituric-acid-reactive substances in diabetes mellitus. *American Journal of Medicine*, 98(5), pp 469-75.

Hisalkar, P.J., Patne, A.B., Karnik, A.C., Fawade, M.M. & Mumbare, S.S. (2012). Ferric reducing ability of plasma with lipid peroxidation in type 2 diabetes. *International Journal of Pharma and Bio Sciences,* 2(2), pp 53-56.

Jamuna Rani, A. & Mythili, S.V. (2014). Study on total antioxidant status in relation to oxidative stress in type 2 diabetes mellitus. Journal of Clinical and *Diagnostic Research*, 8(3), pp 108–110.

Jennings, P.E., McLaren, M. & Scott, N.A., Saniabadi, A.R. & Belch, J.J. (1991). The relationship of oxidative stress to thrombotic tendency in type 1 diabetic patients with retinopathy. *Diabetic Medicine*, 8(9), pp 860-5.

Kesavulu, M.M., Rao, B.K., Giri, R., Vijya, J.S. & Subramanyam, A.C.H. (2001). Lipid peroxidation and antioxidant enzyme status in type 2 diabetics with coronary heart disease. *Diabetes Research and Clinical Practice*, 53(1), pp 33–39.

Krishnan, S., Cozier, Y.C., Rosenberg, L. & Palmer, J.R. (2010). Socioeconomic status and incidence of

type 2 diabetes: results from the Black Women's Health Study. *American Journal of Epidemiology*, 171(5), pp 564–70.

Lee, T.C., Glynn, R.J., Pena, J.M., Paynter, N.P., Conen, D., Ridker, P.M., Pradhan. A.D., Buring, J.E. & Albert, M.A. (2011). Socioeconomic status and incident type 2 diabetes mellitus: data from the Women's Health Study. *PLoS One*, 6(12), e27670.

Lysy, Z., Booth, G.L., Shah, B.R., Austin, P.C., Luo, J. & Lipscombe, L.L. (2013).The impact of income on the incidence of diabetes: a population-based study. *Diabetes Research and Clinical Practice*, 99(3), pp 372–379

National Public Health Survey (1998). Statistics Canada.

Nutthall, S.L., Dunne, F., Kendall, M.J. & Martin, U. (1999). Age-independent oxidative stress in elderly patients with non-insulin-dependent diabetes mellitus. *QJM*, 92(1), pp 33–38.

Rabi, D.M., Edwards, A.L., Southern, D.A., Svenson, L.W, Sargious P.M., Norton, P., Larsen, E.T. & Ghali, W.A. (2006). Association of socio-economic status with diabetes prevalence and utilization of diabetes care services. *BMC Health Services Research*, 6, pp 124.

Raphael, D., Anstice, S., Raine, K., McGannon, K.R., Rizvi, S.K. & Yu, V. (2003). The social determinants of the incidence and management of type 2 diabetes mellitus: are we prepared to rethink our questions and redirect our research activities? *Leadership in Health Services*, 16(3), pp 10–20.

Robbins, J., Vaccarino, V., Zhang, H. & Kasl, S. (2001). Socioeconomic status and type 2 diabetes in African American and Non-Hispanic white women and men: Evidence from the Third National Health and Nutrition Examination Survey. *American Journal of Public Health*, 91(1), pp 76-84.

Satoh, K. (1978). Serum Lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica Chimica Acta*, 90(1), pp 37-43.

Seghrouchni, I., Drai, J., Bannier, E., Riviere, J., Calmard, P., Garcia, I., Orgiazzi, J. & Revol, A. (2002). Oxidative stress parameters in type I, type II and insulin-treated type 2 diabetes mellitus; insulin treatment efficiency. *Clinica Chimica Acta*, 321(1), pp 89-96.

Stelmach, W., Kaczmarczyk – Chalas, K., Bielecki, W. & Drygas, W. (2005). How education, income, control over life and lifestyle contribute to cardiovascular risk factors in adults in a post-communist country. *Public Health*, 119(6), pp 498-508.

Sundaram, R.K., Bhaskar, A., Vijayalingam, S., Viswanathan, M., Mohan, R. & Shanmugasundaram, K.R. (1996). Antioxidant status and lipidperoxidation in type II diabetes mellitus with and without complications. *Clinical Science*, 90(4), pp 255–260.

Velazquez, E., Winocour, P.H., Kesteven, P., Alberti, K.G. & Laker, M.F. (1991). Relation of lipid peroxides to macrovascular disease in type 2 diabetes. *Diabetic Medicine*, 8(8), pp 752-758.

WHO (2017) Diabetes available at, http://who.int/mediacentre/factsheets/fs312/en/index.html.

Yildiz, D., Zeynep, A., Hasan, I. & Tulay, A. (2002). Susceptibility of glutathione and glutathione-related antioxidant activity to hydrogen peroxide in patients with type 2 diabetes: effect of glycemic control. *Clinical Biochemistry*, 35(4), pp 297-301.

Zigler, J.S. & Jr Hess, H.H. (1985). Cataracts in the Royal College of Surgeons rat: evidence for initiation by lipid peroxidation products. *Experimental Eye Research*, 41(1), pp 67–76.