Original Article

JMJ
Jamahiriya Medical Journal
Published by the Libyan Board of Medical Specialities

Reactive Dicarbonyls in Human Cataractous Nuclei

Eman El Mehdi[1], Rashmirekha Sahoo[1], Musa A Ahmed[3], Abir Mohamed rghigh[2], Abdelsalam Al Geilania[3] and Soumendra Sahoo[3].
1-Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Al -Arab Medical Science University, Benghazi,
2- Faculty of pharmacy, Al-fateh university, Tripoli
3-Great River Eye Hospital, Benghazi
Libya

ABSTRACT:

Non-enzymic glycation of long-lived proteins has been implicated in several age related and diabetes related complications, including cataract. Dicarbonyl compounds such as methylglyoxal and glyoxal have been identified as the predominant source for the formation of advanced glycation end products in various tissues including the lens. ***Objective:*** Comparative assessment of the colour of nuclei and quantity of reactive Dicarbonyls present in removed senile cataractous lens nuclei in diabetic and non-diabetic senile cataract cases undergoing routine cataract surgery. ***Methods:*** 30 cases were recruited in this study. The preoperative assessment of types and grading of cataract were done after the cases admitted. All the cases were operated by one surgeon (SS) at Great River Eye Hospital Benghazi. The nucleus of the cataractous lens (core part of the human lens) from operated cases first examined for colour and then transferred in dry state to the laboratory for biochemical assay of reactive dicarbonyls. ***Results:*** Average concentration of detected reactive dicarbonyls in non diabetics was $2.5148x10^{-4} \pm 1.2$ w/w in comparison to $4.566x10^{-4} \pm 1.5$ w/w in diabetics ($p<0.005$) in our study. The average concentration of detected dicarbonyls in nondiabetic females is $2.55X10^{-4} \pm 1.2$w/w.in comparison to$2.45X10^{-4} \pm 1.2$w/w in males, while the detected concentrations in diabetic females are $4.58X10^{-4} \pm 1.4$ in comparison to $4.46X10^{-4} \pm 1.9$w/w in males. ***Conclusion:*** We observed more browning of the removed cataract nuclei in cases that had diabetes in comparison to cases without diabetes. There has been significant high level of reactive dicarbonyls detected in removed nuclei from diabetic individuals than from non-diabetics.

KEY WORDS:

- Methylglyoxal, Advanced glycation End products, 2, 4-Dinitrophenylhydrazine. (JMJ 2008,Vol.8, No.3, 210-212).

INTRODUCTION:

Non-enzymic glycation through Maillard reaction of long-lived proteins has been implicated in several age related and diabetes related complications(1-3). Senile cataract which is usually related to age has now been thought to be caused by excessive oxidative stress(4). It has been shown in experimental studies that the cataract formation has a definite relation to oxidative stress(5). The reactive dicarbonyls like methyl glyoxal and glyoxal usually react with lens protein and are responsible for the browning of crystalline human lenses and accumulation of advanced glycation products(AGE)(6). The formation of AGE owing to glycation may alter the surface charge of the protein, leading to a conformational change that in turn may affect protein-protein and protein-water interactions and ultimately lead to decreased transparency of the eye lens(7-10).

Received 14-03-2008; Accepted 02-06-2008.
Correspondence and reprint request:
Eman El Mehdi
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Al -Arab Medical Science University, Benghazi, Libya.
E-mail:mabuajila@yahoo.com

Spectrophotometry using 2, 4-dinitrophenylhydrazine is a very simple and accurate method of accessing the quantity of reactive dicarbonyls such as Methylglyoxal in various tissues of human beings at 432 nm.(11-13). We undertook a study of assessing the colour of nuclei in removed cataractous lens after its routine surgical removal and subsequently assessing the quantity of reactive diacrbonyls present in those nuclei by visible spectrophotometry.

EQUIPMENTS AND MATERIAL:

Homogenizer PCU speeds: Derhzahler-\kinematic Gmbh/Kriens-(UZERN), (Switzerland). PH-meter: Inolab PH level-1, wissenschaftlich technishe wertstatten, Wellheim (Germany). Water bath: memmert GmbH+Go.KG,Germany. Sonicator: Sonomatic Langford ultrasonics.Centrifuge: Hermle Z300 (Germany). Spectrophotometer: SPECORD 40 Analytica Jena (Germany). Phosphate saline buffered PH 7.4 prepared according to the British Pharmacopeia's-1993. Ethanol; HPLC grade Fisher Scientific UK, HCl: Merk, (Germany).2, 4-dinitrophenylhydrazine: BDH Chemicals Ltd Pool (England).

METHODS:

Total 30 nuclei from senile cataract cases undergoing routine cataract surgery were collected for study. Nuclei collection was made randomly from the cases that had uneventful cataract surgery. The cases were operated by one surgeon (SS) at Great River Eye Hospital, Benghazi, Libya. No modification of surgical procedure was taken because of this study. The nuclei were observed under slit lamp after the surgical removal for amount of pigmentation and then sent for biochemical assay in dry state the same day. The colours of the nuclei were categorized into 3 grades (Grade 1-light yellow, Grade 2-light yellow brown and Grade 3-Dark yellow brown). The nuclei were sent to the laboratory in sterile wide bore bottles without any preservative. The samples were preserved at -190°C till they were assessed spectrophotometrically within a period of 24 hr. This research followed the tenets of the World Medical Association Declaration of Helsinki on ethical principles for medical research involving human subjects.

Assay procedure: The weighed nucleus was taken and stored in 5ml of phosphate saline buffer of pH 7.4 at -19°C. Then each nucleus was homogenized individually in 20 ml of cold phosphate saline buffer of pH 7.4 by PCU speed for 2 minutes. The homogenized mixture was sonicated continuously using ultrasound bath for 5 minutes. Then cooled in ice (00C) for 5 minutes and centrifuged at 4000rpm for 1 hr. 1 ml supernatant and 2.5 ml of 2, 4-dinitrophenylhydrazine were mixed in a closed test tube and heated in water bath at 42 0 C for 40 minutes. Absorbance was measured at 432 nm by a double beam spectrophotometer (11).

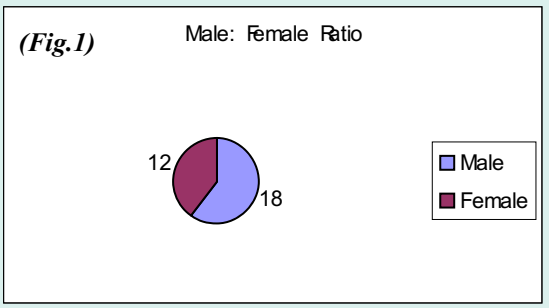
RESULTS:

In our recruited 30 cases for study 18 were males and the remaining 12 were females (Fig.1). The mean age group in non-diabetic cases was 51±10 years whereas in the diabetic group was 54±10 years. Nine cases had variable grades of diabetes. Out of the 30 cases around 8 cases were pigmented hard cataracts the rest 22 were white and soft cataract. All cases had uncomplicated cataract surgery by the surgeon. The average duration of diabetes was 6 years. All cases were taking oral antidiabetic treatment. The nuclei colour grading in non diabetic as well as the diabetic group has been plotted on a bar diagram which showed more browning of nuclei removed from diabetic cases. Around 9% of removed nuclei from nondiabetic individuals had dark yellow brown as against 33% removed nuclei from senile cataract cases that had associated diabetes (Fig.2).

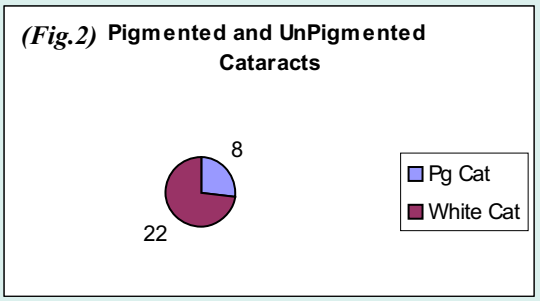
NON DIABETIC GROUP VS DIABETIC GROUP

The reactive dicarbonyls concentration in removed nuclei of cataractous lenses in nondiabetics were found to be $2.5148x10^{-4}$± 1.2 w/w where as in diabetic group it was an average concentration of $4.566x10^{-4}$±1.5 w/w.(Fig.3).

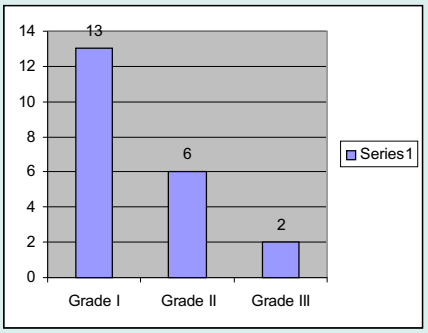
The average concentration of detected dicarbonyls in nondiabetic females was $2.55X10^{-4}$±1.2w/w.in comparison to$2.45X10^{-4}$±1.2w/w in non diabetic males, while the average detected concentrations in diabetic females was $4.58X10^{-4}$±1.4 in comparison to 4.46X10±1.9w/w in diabetic males (Fig.4,5).



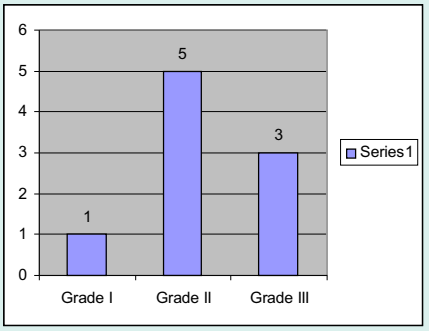
(Fig.1)



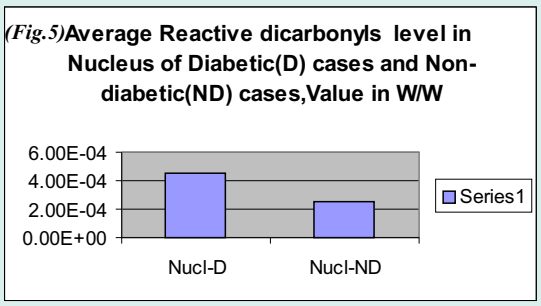
(Fig.2)



(Fig.3)The non Diabetic Group.



(Fig.4)The Diabetic Group.



DISCUSSION:

In this study we have chosen to take the nuclei from senile cataract cases between the age group 40 to 65 years. Though the cases were randomly selected, but there was no statistically significance in age group difference in the two groups.

Its evident from our observation that the nuclei removed from diabetic cases are more pigmented in comparison to those removed from nondiabetic individuals ($p<0.005$). This might be due to high level of dicarbonyls which are responsible for browning of the lens(6,14). The concentrations of reactive dicarbonyls are significantly higher in diabetic nuclei than from nondiabetics ($p<0.005$). But in this study the reactive dicarbonyl levels do not show any statistical difference ($p>0.5$) between the nuclei removed from males in comparison to those from females in both diabetic and non diabetic groups. The high value of reactive dicarbonyls in cataractous nuclei of diabetics suggests that senile population with diabetes are more prone to develop cataract in comparison to those without diabetes, and also may suggest that the lowering of dicarbonyls by pharmaceutical medications (e.g. antioxidant) might help in reducing cataract formation and progress. These will be discussed in a further extended study which is under way.

CONCLUSION:

The present study indicates a more brown colouration of the removed diabetic cataract nuclei in comparison to nondiabetic cases. There is a significantly higher level of reactive dicarbonyls concentrations detected in removed nuclei from diabetic individuals than from nondiabetics. No significant variations were observed between females and males in both diabetic and non-diabetics sample.

REFERENCES:

1-Thornalley PJ, Hooper NI, Jennings PE. The human red blood cell glyoxalase system in diabetes mellitus. **Diabetes Res Clin Pract. 1989: 1: 7(2):115-20**.

2-Vlassara H.J. Recent progress on the biologic and clinical significance of advanced glycosylation end products. **Lab. Clin. Med. 1994:124: 19-30**.

3-Thorpe S. R and Baynes, J. W. Role of the Maillard reaction in diabetes mellitus and diseases of aging. **Drugs Aging. 1996: 9(2): 69-77**.

4-Sungchur M, Rohan F, Marjorie F L. Induction of thioltransferase and thioredoxin/ thioredoxin reductase systems in cultured porcine lenses under oxidative stress. **Invest. Ophthalmol Vis Sci. 2005: 46 (10): 3783-9**.

5-Kyselova Z,Garcio.S, Gajdosikova. A,Gajdosik.P,Stefek. M.Temporary relations between lens protein oxidation and cataract development in streptozotocin induced diabetic rats. **Physiol. Res. 2005: 54: 49-56**.

6-Nagaraj. R.. H , Oya I. T, Padayatti.P.S. Enhancement of chaperone function of a-crystallin by methylglyoxal modification,
Biochem. 2003:42:10746-10755.

7-Beswick H. T, and Harding J. J. Conformational changes induced in lens a- and g-crystallins by modification with glucose 6-phosphate. Implications for cataract. **Biochem. J. 1987: 246: 761-769**.

8-Crabbe M. J. Cataract as a conformational disease - the Maillard reaction, a-crystallin and chemotherapy. **Mol. Biol. 1998: 44: 1047-1050.**

9-Liang J. N, and Rossi M. In vitro non-enzymatic glycation and formation of browning products in the bovine lens a-crystallin **Exp Eye Res. 1990: 50: 367-371**.

10-Haik GM, Jr Lo T.W.C, Thornalley PJ. Methylglyoxal concentration and glyoxalase activities in the human lens. **Exp Eye Res. 1994:59:497-500**.

11-Gilbert R P, Brandt R B.Spectrophotometric determination of
methylyglyoxal with 2, 4- dinitrophenylhydrazine. **Analytical Chemistry, 1975: 47: No 14.2418-22.**

12-Davidson A.G, Beckett A.H, Stenlake J.B. Ultraviolet-Visible absorption Spectrophotometry. Practical Pharmaceutical Chemistry 4th Edition, Part Two. **The Atlone Press, London, UK, 1988, 275-337**.

13-Ewing G.W, The absorption of Radiation. Ultraviolet and visible. In : Instrumental Methods of Chemical Analysis 4th edition. **McGraw-Hill Book. New York, USA, 1975, 34-84**.

14-Satish M, Kumar P, Redday Y. Effect of dicarbonyl-induced browning on a-crystallin chaperone-like activity: Physiological significance and caveats of in vitro aggregation assays. **Biochem. J. 2004: 379: 273-282.**