



Original Research Article

Association of oxidative stress with the bone turnover in pre and post menopausal diabetic women

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ARTICLE INFO

Article history:

Received 17-02-2020

Accepted 13-04-2020

Available online 30-06-2020

Keywords:

Osteocalcin

TRAP

Antioxidants

Lipid peroxidation

Pre menopausal

Post menopausal

Diabetes

ABSTRACT

Diabetes is today a worldwide problem because of its global prevalence. India is also one of the hub. The long term complications of diabetes have been extensively studied but it has been observed that diabetes also affects the bone health. The relation of Bone health, Oxidative stress and diabetes has not been looked into to a great extent.

Objective: To study the relation of Bone health, Oxidative stress in diabetic individuals.

Research Design and Methods: 330 individuals were included in the study. Dividing into four group i.e group I included Pre Menopausal type II DM (n=80), group II was age matched control (n=75), group III were Post Menopausal type II DM (n=100) and group IV (n=75) were age matched Post Menopausal control group. The protocol of the study was approved by ethical committee of the institute. GHbA1C, Osteocalcin, TRAP, Alkaline Phosphatase, SOD, GSH, GPx, Gred, MDA and Minerals like Calcium, Phosphorous, Magnesium were estimated.

Results: A highly significant increase ($p < 0.0001$) in levels of MDA and antioxidants (SOD, GSH, GPx, Gred) was observed in the premenopausal women while through MDA levels were high in the menopausal women. While the altered levels of the bone markers were observed. Sr TRAP as the marker of bone resorption was increased significantly ($p < 0.0001$) in pre but not significant in the post menopausal group while Sr Osteocalcin and Sr Alkaline phosphatase markers of bone formation was low in pre and not significant variation observed in the post menopausal women.

Conclusion: The Oxidative stress, hyperglycemia and the decreased antioxidant defense leads to enhanced osteoclastic activity and a decreased osteoblastic activity in pre and post menopausal diabetic women.

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1. Introduction

Diabetes is today a worldwide problem. The global prevalence of diabetes is estimated to increase still further, it is projected that by 2025 there will be 380 million people with type 2 diabetes and 418 million people with impaired glucose tolerance¹ and India the situation is going to be more difficult as it has been reported that by 2025 India will be the hub of diabetes. People with diabetes are also at increased risk of developing cardiovascular, peripheral vascular and cerebrovascular disease. Hence this disease is also called as a “silent killer”.^{2,3}

But a new emerging complication is the impact of the disease on the Bone health. It has been observed that diabetic patients are at a greater risk of fracture due to poor quality of the bones. At the same time, healing is delayed in diabetes, including nonunion. Bone has been known to be required for three classical functions of Organ protection, locomotion, and calcium–phosphorus homeostasis. There is a constant renewing by remodeling which helps in the repair of the microdamage and participates in fracture healing. This remodeling is effected in the diabetic individuals due to hyperglycemia.⁴ These observations evoked interest to conduct this study because women generally neglect their health and hence it was interesting to analyze the bone health in Indian Diabetic women.

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2. Materials and Methods

The study enrolled 330 individuals i.e 180 Diabetic subjects (group I and III) and control group comprising of 150 healthy subjects (group II & IV). Dividing into four group i.e group I included Pre Menopausal with type II DM (n=80), group II was age matched control (n=75), group III were Post Menopausal with type II DM (n=100) and group IV (n=75) were age matched Post Menopausal control group. No subject was included who had a previous history of stroke, myocardial infarction or any other cardiovascular disease. The study protocol was approved by the Ethics Committee of the Institution and all the subjects included in the study volunteered after proper consent taken. All ethical norms were followed during the study.

2.1. Collection of specimen

After 12 hrs of fast, venous blood sample were collected in different bulbs under aseptic conditions. EDTA bulb was used for glycosylated hemoglobin estimation by resin binding method.⁵ Plain bulb was used for estimation of serum Calcium, Phosphorous, Magnesium. Glycosylated hemoglobin, TRAP, Osteocalcin, Serum Alkaline Phosphatase, Serum Calcium, Serum Phosphorous were estimated by commercially available kit. While Serum Magnesium,⁶ Superoxide dismutase (SOD) by Marklund and Marklund,⁷ malondialdehyde (MDA) by Wilbur K. M et al.⁸ Acid citrate bulb was used for erythrocyte reduced glutathione(GSH) measurement by method of Beutler et al.⁹

2.2. Statistical analysis

Students “t” test was used to compare the continuous variables between groups. Pearson’s correlation was employed to calculate the ‘r’ value. Statistical significance was defined as $p < 0.05$. The statistical analysis was done using SPSS version 14.0

3. Results

Table 1 shows that HbA1C is very significantly increased ($p < 0.0001$) in group I & III as they are diabetic subjects with type II Pre and Post menopausal diabetic women respectively.

Table 2 shows, serum MDA parameter of lipid peroxidation and antioxidants like GSH, GPx, G red and SOD. MDA levels have been very significantly increased ($p < 0.0001$) in the group I & III DM subjects than the respective control groups. While the antioxidants show an interesting trend in group I i.e Pre Menopausal women but the antioxidant levels were not significant in group III even when MDA levels were very high. The levels of SOD have been very significantly decreased ($p < 0.0001$) in the Post menopausal diabetic women than the control group while a no significant variation could be observed in the levels of

GSH, GPx, G red when compared to the control.

Table 3 indicates the Bone minerals, Osteoblastic and Osteoclastic activity in various groups. It has observed that the Serum Calcium and Serum Magnesium levels were significantly low in the premenopausal diabetic women. The Osteoblastic activity (i.e measured by Sr. Alkaline Phosphatase and Osteocalcin) was significantly low ($p < 0.0001$) while Osteoclastic activity (Sr. TRAP) was found to be significantly increased ($p < 0.0001$) in the Pre Menopausal diabetic group.

4. Discussion

In the present study, we have observed an increased ($p < 0.0001$) level of glycosylated hemoglobin which was used as an index of metabolic control in the study group in both pre and post menopausal diabetic women (Table 1). It is this “glucose toxicity” which leads to cellular dysfunction that become irreversible over a period of time. Hyperglycemia induces increased synthesis of reactive oxygen species via oxidative phosphorylation during anaerobic glycolysis, via the Schiff reaction during glycation, via glucose autoxidation, and via hexosamine metabolism under supraphysiological glucose concentrations. In the present study as per indicated in Table 2 an increased level of MDA was observed in comparison to control ($p < 0.0001$). This is in accordance with previous finding that hyperglycemia induces over production of oxygen free radicals in diabetes.^{10,11} In the present study it was observed that in the premenopausal women though had a raised MDA levels (6.75 ± 2.42) indicating oxidative stress but it was getting counter balanced by the increased level of antioxidants i.e GSH, GPx, Gred, SOD while in the post menopausal women the levels of the GSH, GPx, Gred were not increased through an oxidative stress was observed (MDA level 8.03 ± 1.28). This might be due to the prolonged exposure to hyperglycemia which inactivates the enzymes.^{11,12} The levels of SOD was observed to be significantly low ($p < 0.0001$) in group III when compared with group IV indicating “Oxidative stress” which has been observed by other researcher.¹³ Thus indicating that the combined effect of Oxidative stress and depleted antioxidant defense system causes oxidative injury. The result of the present study indicates that hyperglycemia caused increased production of free radical (Table 4) as a significant positive correlation ($p < 0.0001$) between glycosylated hemoglobin and MDA in pre and post menopausal women. While a significant negative correlation ($p < 0.0001$) observed between the various antioxidants with MDA only in group I while this is through indicating negative correlation but not significant.

The levels of the bone minerals i.e Sr. Calcium showed a significant change ($p < 0.001$) in pre menopausal women but the Sr. Phosphorous levels were unaltered in all the study subjects. At the same time levels of Sr Magnesium

Table 1: Clinical and biochemical parameters in diabetic patients (type I & II) as compared to control.

Parameters	Group I	Group II	P Value	Group III	Group IV	P Value
	Mean ± SD (n=80)	Mean ± SD (n=75)		Mean ± SD (n=100)	Mean ± SD (n=75)	
Age (Yrs)	45 ± 7	37.1 ± 9		62 ± 7	61 ± 6	
Ghb(%)	8.3 ± 1.45	4.9 ± 0.39	<0.0001	10.23 ± 2.83	5.2 ± 0.9	<0.0001

Table 2: Oxidative and antioxidant parameters in diabetic patients (type I & II) as compared to control:

Parameters	Group I	Group II	P Value	Group III	Group IV	P Value
	Mean ± SD (n=80)	Mean ± SD (n=75)		Mean ± SD (n=100)	Mean ± SD (n=75)	
MDA(nmol/ml)	6.75 ± 2.42	1.66 ± 1.34	<0.0001	8.03 ± 1.28	3.66 ± 1.12	<0.0001
Erythrocyte GSH(μmol/g of Hb)	8.91 ± 5.37	2.63 ± 1.21	<0.0001	2.90 ± 1.14	2.91 ± 1.65	>0.01
SOD(units/ml)	9.2 ± 2.95	3.2 ± 1.49	<0.0001	2.14 ± 1.47	5.85 ± 1.89	<0.001
GPx (μmol/l/min)	189.22 ± 7.9	101.22 ± 2.21	<0.0001	99.32 ± 4.14	101 ± 1.65	>0.01
G red (μmol/l/min)	8.65 ± 1.2	2.56 ± 1.5	<0.0001	4.94 ± 1.47	4.85 ± 2.89	>0.01

Table 3: Data of the Bone Minerals and levels of Osteoblastic and Osteoclastic activity in various groups:

Parameters	Group I	Group II	p Value	Group III	Group IV	p Value
	Mean ± SD (n=80)	Mean ± SD (n=75)		Mean ± SD (n=100)	Mean ± SD (n=75)	
Calcium (mg%)	8.9 ± 0.5	9.5 ± 1.1	<0.01	8.7 ± 0.65	9.2 ± 0.9	>0.01
Phosphorous (mg%)	2.9 ± 0.6	3.0 ± 0.51	>0.01	2.7 ± 0.7	2.9 ± 0.5	>0.01
Magnesium (mg%)	2 ± 0.4	3.12 ± 0.32	<0.0001	1.4 ± 0.8	2.8 ± 0.3	<0.001
Alkaline Phosphatase (IU/L)	48.53 ± 8.91	90.26 ± 5.44	<0.0001	29.79 ± 7.04	47.91 ± 4.72	<0.01
Osteocalcin (ng/ml)	3.1 ± 1.3	11.31 ± 6.09	<0.0001	3.4 ± 1.5	7.4 ± 4.4	<0.0001*
TRAP(IU/L)	3.4 ± 1.3	0.73 ± 0.43	<0.0001	3.5 ± 3.2	1.6 ± 0.8	<0.01

Table 4: Correlation between MDA and various parameters in each group

Correlation between MDA	Group I		Group II		Group III		Group IV	
	r Value	P Value	r Value	P Value	r Value	P Value	r Value	P Value
GSH	-0.23	<0.001	-0.23	<0.001	0.19	>0.05	0.04	>0.05
G Red	-0.64	<0.001	-0.5	>0.05	0.07	>0.05	-0.1	>0.05
G Px	-0.68	<0.001	-0.36	<0.001	-0.13	>0.05	-0.14	>0.05
SOD	-0.43	<0.0001	-0.42	<0.0001	-0.12	>0.05	-0.04	>0.05
GHb	0.45	<0.0001	0.28	<0.05	0.97	<0.0001	0.99	<0.0001
Alkaline Phosphatase	-0.51	<0.0001	-0.51	<0.0001	0.07	>0.05	-0.05	>0.05
TRAP	0.4	<0.001	0.47	<0.001	-0.10	<0.0001	-0.14	>0.05
Osteocalcin	-0.38	<0.001	-0.34	<0.001	-0.28	<0.0001	-0.3	>0.05

showed a decrease in the premenopausal ($p < 0.0001$) and post menopausal women ($p < 0.001$).

On the other hand hyperglycemia and Oxidative stress both are said to have a crucial role in bone pathophysiology.¹⁴ The current study used Sr TRAP as the marker of bone resorption while Sr Osteocalcin and Sr Alkaline phosphatase as bone formation. In our study the level of TRAP was significantly high ($p < 0.0001$) in the post and pre menopausal diabetic women than the respective control at the same time markers of bone formation are indeed decreased similar to other study done.^{15–18} But the levels of Sr. Alkaline phosphatase was slightly decreased. This pattern of changes suggests that an extent of imbalance of bone resorption and bone formation does occur in the diabetic individual though there are other factors that govern it. ($p < 0.0001$). When this is clubbed with the decreased magnesium levels it can be understood that magnesium is an important factor of the bone matrix which determine the bone fragility. Hence magnesium depletion can affects all stages of skeletal metabolism adversely, leading to cessation of bone growth, decreased osteoblastic and osteoclastic activity, osteopenia and bone fragility.^{19,20}

5. Conclusion

All this indicates that hyperglycemia over a period of time results in Oxidative stress and affects the bone health in the diabetic women. The body mechanism is able to resist this damage by the antioxidants in the pre menopausal age but as menopause sets it the risk of bone fragility is observed to be increased. Hence the risk of fracture is increased. So care should be taken to monitor and maintain the blood sugar levels.

6. Source of Funding

None.

7. Conflict of Interest

No conflict of interest to disclose.

References

1. van DS, Beulens J, van der SYT, Grobbee DE, Neal B. The global burden of diabetes and its complications: an emerging pandemic. *Eur J Cardiovasc Prev Rehabil.* 2010;17(1_suppl):s3–s8.
2. Misra A, Pandey RM, Devi JR, Sharma R, Vikram NK, Khanna N. High prevalence of diabetes, obesity and dyslipidaemia in urban slum population in northern India. *Int J Obesit.* 2001;25(11):1722–9.
3. Premealatha G, Shanthirani S, Deepa R, Markovitz J, Mohan M. Prevalence and risk factors of peripheral vascular disease in a selected south Indian population. *Diabetes Care.* 2000;23:1295–1300.

4. Confavreux CB. Bone: from a reservoir of minerals to a regulator of energy metabolism. *Kidney Int.* 2011;79(S121):S14–9.
5. Trivelli LA, Ranney HM, Lai HT. Hemoglobin Components in Patients with Diabetes Mellitus. *New Engl J Med.* 1971;284(7):353–7.
6. Paolisso G. Hypertension, Diabetes Mellitus, and Insulin Resistance The Role of Intracellular Magnesium. *Am J Hypertens.* 1997;10(3):346–55.
7. Marklund S, Marklund G. A simple assay for Super Oxide Dismutase using autooxidation of pyrogallol. *Eur J Bio Chem.* 1974;47:469–72.
8. Wilbur KM, & Mernhein, Shapior OW. The thiobarbituric acid method for malondialdehyde estimation. *Arch Biochem Biophys.* 1943;250:305–13.
9. Beutler E, Duran O, Kelley BM. Improved method for the determination of blood glutathione. *J Lab Clin Med.* 1963;61:882–8.
10. Sakai K, Matsumoto K, Nishikawa T, Suefuji M, Nakamaru K, Hirashima Y, et al. Mitochondrial reactive oxygen species reduce insulin secretion by pancreatic β -cells. *Biochem Biophys Res Commun.* 2003;300(1):216–22.
11. Oates PJ, Mylari BL. Aldose reductase inhibitors: therapeutic implications for diabetic complications. *Expert Opin Investig Drugs.* 1999;8(12):2095–2119.
12. Pillai-Nair S, Shah NC. Alteration in enzymatic antioxidant defense in diabetes mellitus. *Biomed Res.* 2012;23(3):402–4.
13. Greene DA, Stevens MJ, Obrosova I, Feldman EL. Glucose-induced oxidative stress and programmed cell death in diabetic neuropathy. *Eur J Pharmacol.* 1999;375(1–3):217–23.
14. Wauquier F, Leotoing L, Coxam V, Guicheux J, Wittrant Y. Oxidative stress in bone remodelling and disease. *Trends Mol Med.* 2009;15(10):468–77.
15. Suzuki K, Ishida H, Takeshita N, Taguchi Y, Sugimoto C, Nosaka K, et al. Circulating Levels of Tartrate-Resistant Acid Phosphatase in Rat Models of Non-Insulin-Dependent Diabetes Mellitus. *J Diabetes Complications.* 1998;12(3):176–80.
16. Fernández-Real JM, Ricart W. Osteocalcin: a new link between bone and energy metabolism. Some evolutionary clues. *Curr Opin Clin Nutr Metab Care.* 2011;14:360–6.
17. Schmidt-Gayk H, Roth HJ, Becker S, Reichel H, Boneth HG, Knuth UA. Review Alkaline phosphatase and tartrate-resistant acid phosphatase in osteoblasts of normal and pathologic bone. *Bonucci E, Nanci A. Ital J Anat Embryol.* 2001;106(2):129–33.
18. Varma M, Paneri S, Badi P. Correlative study of bone related Biochemical parameters in normal post menopausal women and hyperglycaemic post menopausal women. *Biomed Res.* 2005;16(2):3–5.
19. Wallach S. Effects of magnesium on skeletal metabolism. *J Am Coll Nutr.* 1989;8:457.
20. Villegas R, Gao Y. Dietary calcium and magnesium intakes and the risk of type 2 diabetes: the Shanghai Women's Health Study. *Am J Clin Nutr.* 2009;89(4):1059–67.

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Cite this article: Nair SP, Shah NC. Association of oxidative stress with the bone turnover in pre and post menopausal diabetic women. *Int J Clin Biochem Res* 2020;7(2):181-184.