International Journal of Research in Medical Sciences
Palem SP. Int J Res Med Sci. 2017 Jul;5(7):3114-3119
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20172998

Original Research Article

HbA1c is a risk factor for cardiovascular disease in association with oxidative stress in patients with type 2 diabetes mellitus

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Received: 29 April 2017
Accepted: 24 May 2017

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ABSTRACT

Background: Cardiovascular disease (CVD) is a major cause of death in diabetic subjects. Glycaemic status is one of the vital factor involved in vascular complications. It was clear the effect of glycaemia on microvascular complications, but uncertain on macrovascular complications. As we know oxidative stress plays a major role in the development of atherosclerosis and cardiovascular disease. Since oxidative stress is a risk factor for cardiovascular disease, the study has designed to perceive an association between HbA1c and oxidative stress in patients with type 2 diabetes mellitus for early prediction of cardiovascular events.
Methods: 120 subjects were taken into the study, among these 60 type 2 diabetic subjects and remaining 60 subjects were healthy controls. The parameters like HbA1c, MDA and FRAP were estimated by established methods. 'Kruskal Wallis' test was used for variables in the parameters and Pearson correlation test was used to perform correlation between HbA1c and oxidative stress.
Results: High level of HbA1c and MDA, low level of FRAP were found in patients with type 2 diabetes than healthy controls. The study was also found HbA1c have positive association with malondialdehyde (MDA) and negative association with FRAP.
Conclusions: HbA1c was positively associated with oxidative stress in patients with type 2 diabetes mellitus. In this scenario, type 2 diabetic patients with high level of HbA1c might have risk of cardiovascular events.

Keywords: CVD, Ferric reducing ability of plasma, Glycosylated hemoglobin, MDA, Type 2 diabetes mellitus

INTRODUCTION

Cardiovascular disease is the major cause of mortality in patients with both type 1 and type 2 diabetes mellitus.[1] The risk of cardiovascular events is two to four times more in diabetic subjects compared to general population. Some of the studies have been reported that glycaemia was associated with cardiovascular disease and death in general population with and without diabetes.[2,3] Among diabetic patients glycaemic control may be an important factor for predicting cardiovascular events.[2]

Last two decades it has clear that reactive oxygen species, including free radicals are involved in cardiovascular disease. In current days, clinical importance of oxidants is growing interestingly.[4] Oxidative stress is frequently involved in the pathogenesis and development of cardiovascular diseases including hypertension, dyslipidemia, diabetes mellitus, atherosclerosis, myocardial infarction and heart failure.[5,6]

A meta-analysis study has been reported that 18% greater risk of cardiovascular events in patients with every 1% higher level of HbA1c. While some of the large-scale clinical trials failed to demonstrate beneficial effect of near-normal HbA1c on major cardiovascular events and death. Thus, it is unclear that whether HbA1c levels were involved in cardiovascular events and mortality in

patients with diabetes mellitus.[3] Since, Oxidative stress is an initiator for pathogenesis and development of cardiovascular disease. The study has designed to perceive an association between HbA1c and oxidation stress in patients with type 2 diabetes mellitus for early prediction of cardiovascular events.

METHODS

The study was conducted on 120 subjects; among these 60 were type 2 diabetes mellitus subjects and remaining 60 were healthy subjects. Type 2 diabetic subjects were selected from Vinayaka Missions Kirupananda Variyar Medical College and Hospital at Salem. Type 2 diabetic patients without any complications were included in this study. The blood samples were collected from patients after ethical clearance from institutional ethical committee. 4 ml of blood sample was collected after overnight fasting of 12 hours from each patient and 1ml transferred in fluoride tube for blood sugar, 1 ml in EDTA tube for HbA1c, 2 ml in heparin tube for MDA and FRAP. 1 ml of post-prandial blood sugar was collected after 2 hours of breakfast from each subject. Blood sugar was estimated by GOD-POD method, HbA1c was estimated by immunotubidimetric method in auto-analyzer. MDA was measured by TBARS (Thiobarbituric acid reactive substances) method and total antioxidant capacity was estimated as FRAP in spectrophotometer.

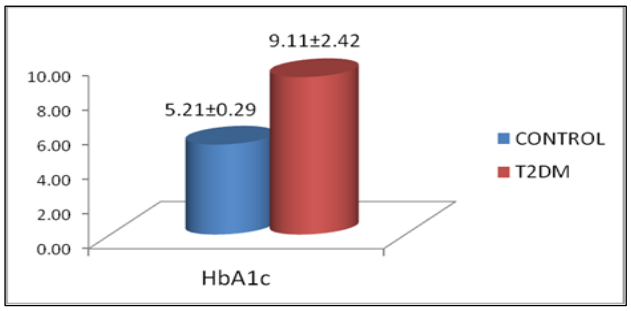
Statistical analysis

Statistical analysis was done by using SPSS software. "Kruskal Wallis" test was performed to analyze significant variables of parameters between the study groups. "Pearson correlation" test was used to perform correlation between HbA1c and oxidative stress. P<0.05 was considered as statistically significant.

RESULTS

In our study, Table 1 shows significantly high level of BMI, fasting and postprandial blood sugar in type 2 diabetic subjects (24.45±3.11, 159.85±68.49, 277.98±85.90) compared to healthy controls (20.48±0.81, 87.62±9.95, 119.65±6.07).

Significantly high level of HbA1c (9.11±2.42) was observed in type 2 diabetic subjects than healthy controls (5.21±0.29). P<0.05 considered as statistically significant (Figure 1).



Data expressed as Mean±SD, statistical analysis was performed by "Kruskal Wallis" test. *Significant at 'p' value <0.05.

Figure 1: HbA1c level between the study groups.

Figure 2 shows significantly high level of MDA (2.48±1.70) and lower level of FRAP (0.70±0.10) were identified in type 2 diabetic subjects compared to healthy controls (0.72±0.17, 0.91±0.30). The study was also found, HbA1c have positive correlation with MDA and negative correlation with FRAP (Table 2).

Table 1: Clinical characteristics of the study subjects.

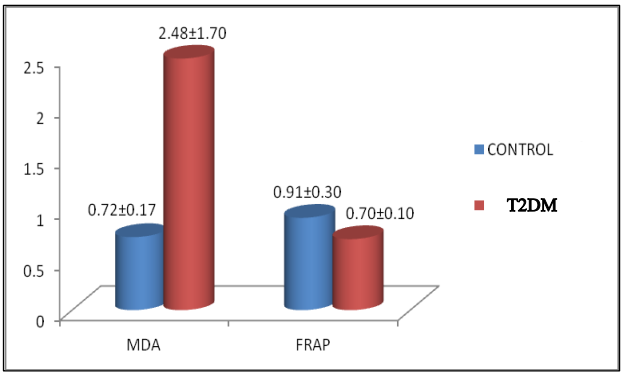
Parameters	Control (n-60)	T2DM (n-60)	p values
Age	49.30±10.27	50.85±10.23	>0.05
BMI (kg/m^2)	20.48±0.81	24.45±3.11	<0.05*
FBS (mg/dl)	87.62±9.95	159.85±68.49	<0.05*
PPBS (mg/dl)	119.65±6.07	277.98±85.90	<0.05*

Data expressed as Mean±SD, Statistical analysis was performed by "Kruskal Wallis" test. *Significant at 'p' value <0.05.

Table 2: Association between HbA1c and oxidative stress.

Parameters	HbA1c	
	'r' Value	'p' Value
MDA	0.466	0.000**
FRAP	-0.315	0.001**

Statistical correlation was performed by "Pearson correlation" test.



Data expressed as Mean±SD, Statistical analysis was performed by "Kruskal Wallis" test. *Significant at 'p' value <0.05.

Figure 2: Comparison of oxidant/ antioxidant markers between study groups.

DISCUSSION

HbA1c and oxidative stress are important factors seen in type 2 diabetes mellitus. Oxidative stress is common in diabetic subjects and HbA1c can be seen in uncontrolled diabetic subjects. The treatment for type 2 diabetes mellitus is targeted to reduce the risk of diabetic complications in patients. The intensive treatment was moderately reduces the risk of microvascular complications, but benefits of macrovascular complications are less clear.[7] Biochemical, physiological and pharmacological data were supported a link between free radicals and cardiovascular tissue injury. The major vascular risk factors include hypertension, dyslipidaemia, diabetes and smoking and these factors are associated with increase production of ROS in vascular tissues.[4]

Body mass index (BMI)

The present study has shown significantly high level of BMI in patients with type 2 diabetic subjects compared to control subjects (Table 1). Ganz, et al has reported that individual type 2 diabetic subjects have higher level of BMI compared to control and it also shown an association between increased BMI and overweight or obese.[8] The main cause of weight gain in type 2 diabetes is due to excess of calorie intake, physical inactivity which has revealed by Hollanderl.[9] Weight gain is a well-known factor for adversely effect of cardiovascular risk.[10]

Insulin is a major hormone which effects on metabolism in the determination of body weight. Increased Insulin sensitivity has been associated with the expansion of adipose tissue in the body; the reason behind this is increased action of insulin on muscle and liver.[11] Insulin suppresses the lipase action and reduced lipolysis, finally increase the deposition of fat in adipose tissue and leads to weight gain. Development of Insulin resistance is an adaptive physiological mechanism to prevent the additional weight gain due to the enhancing insulin action in adipose tissue.[12]

Most of the prospective studies have been reported an association between BMI and CVD.[13-15] Song and Hardisty has found that type 2 diabetic patients have higher risk of cardiovascular disease with irrespective difference in the level of BMI.[16] There is evidence that individual type 2 diabetic patients are at risk of CVD and mortality in both obese and non-obese subjects.[17,18] BMI is considered as a risk factor for T2DM and cardiovascular disease; it shows that once diabetes develops other factors are more important in the development of CVD. UKPDS has not considered obesity as a risk factor for the development of CVD in type 2 diabetes.[19] Hence, BMI cannot be considered as an independent risk factor for cardiovascular disease in diabetic subjects.

Glycosylated hemoglobin (HbA1c)

Graph 1 show that patients with type 2 diabetes have high level of HbA1c compared to healthy control. Nathan et al. has reported a significantly high level of HbA1c in both type 1 and type 2 diabetes compared to non-diabetic subjects, and it has also shown an association between HbA1c and mean glucose level.[20] Moreover, the earlier study has identified an increase level of HbA1c along with increased mean blood glucose level in patients with diabetes than non-diabetes. Since, HbA1c is a compound synthesized from non-enzymatic reaction of glucose and hemoglobin, especially in uncontrolled diabetic patients.[21] Hence, HbA1c was associated with fasting and post-prandial blood sugar in diabetic patients.[22] ACCORD trial had shown an increased hypoglycemia with poor glycaemic control compared with additional popular HbA1c level in type 2 diabetes.[23] Another study has reported that high level of HbA1c mean in hypoglycemic subjects compared with non-hypoglycemic subjects. The same study also shown diabetic patients with <6% and >9% of HbA1c values tended to be a higher risk of hypoglycemia compared to HbA1c value (7-7.9%) range.[7] In our study, poor glycaemic control might be the reason for high level of HbA1c in type 2 diabetic subjects.

HbA1c and cardiovascular risk

A prospective study has shown significantly elevated levels of HbA1c in patients with coronary artery disease (CAD) and it also found the severity of coronary lesions in CAD patients. However, the study also reported that predictable power of HbA1c exist only in diabetic patients with stable CAD, but not in subjects without diabetes. This shows the important role of HbA1c to predict CAD in diabetic subjects.[24] However, other studies have reported an association of high normal glucose and HbA1c level with the incidence of CAD in individual patients with or without diabetes.[25,26] Ikeda F et al study has suggested that increased HbA1c levels can be an independent risk factor for CVD, especially CHD (coronary heart disease) and ischemic stroke. But, HbA1c

can be additional strong factor along with traditional risk factors for the prediction of CVD.[27]

Seven years of fallow-up study on 2,851 diabetic subjects, 119 subjects has shown the development of cardiovascular disease (CVD) with significant increase in the level of HbA1c (5.5-6.4 and 6.5).[27] Veterans affairs diabetes trial (VADT) study has reported that 38% of type 2 diabetic patients had CVD risk at baseline by using coronary artery calcium (CAC) as measure of atherosclerosis. Good glycaemic control (HbA1c) in diabetic patients with CAC value below 100 was related to the future risk of cardiovascular events, but this effect was not seen in diabetic patients with higher CAC value.[28] Bots SH et al study has shown a significant relation of HbA1c with the risk of all-cause mortality in diabetic subjects, but not to the risk of cardiovascular events.[29]

However, an earlier study has reported that HbA1c was not associated with the prevalence of cardiovascular disease in patients with diabetes.[30] In another study has shown significant association of HbA1c with severity of coronary atherosclerosis in selected men's undergoing coronary angiography.[31] Nevertheless, HbA1c alone is not a factor to predict cardiovascular risk, but elevated level of HbA1c clustered with other risk factors in people without diabetes and indicates additional risk factor to predict CVD. The increased level of HbA1c may be mediated by the clustering of hypertension, dyslipidemia, hyperglycemia and smoking. Hence, HbA1c can be an additional predictable risk factor for CVD along with other risk factors.[32]

Oxidative stress in type 2 diabetic subjects

Free radicals may have a large in the pathogenesis of many diseases such as diabetes mellitus, atherosclerosis and myocardial infarction. Free radical targets biological molecules, especially lipid oxidation. Malondialdehyde is the end product of lipid peroxidation commonly used to determine the oxidant/antioxidant balance in diabetic subjects.[33] In our study, Graph 2 shows significantly high level of MDA and lower level of FRAP (antioxidant power) in patients with type 2 diabetes compared to healthy controls. Earlier studies have been shown a significantly increased level of MDA and decreased total antioxidants levels in type 2 diabetes mellitus.[34,35] Experimental studies have observed the alterations of proteins and increase lipid peroxidation in diabetes mellitus, high glucose level may be the reason to stimulate platelet aggregation and auto-oxidation of glucose and produces free radicals in diabetic subjects.[33,36,37]

Glucose oxidation has been believed to be the main reason of free radicals. Glucose is oxidized to an enediol radical anion that is further converted into reactive ketoaldehydes and to superoxide anion radicals. The superoxide anion radicals cannot undergo dismutation due to the deficiency of catalase or glutathione peroxidase enzyme. Therefore, hyperglycemia can promote lipid peroxidation of low density lipoprotein (LDL) by superoxide dependent pathway leads to produce free radicals.[38] This might be the reason behind increased oxidative stress and simultaneously decline of anti-oxidant defense system along with high level of glucose in type 2 diabetic patients. Reactive oxygen species generation is the main pathophysiological mechanism linked with glucose metabolism to endothelial dysfunction and atherosclerosis. Hence, hyperglycemia plays an important role in causing vascular complications in diabetes.[39]

Oxidative stress and cardiovascular disease

Increased oxidative stress may play a major role in the development of diabetic vascular complications. Oxidative stress induces cell proliferation, hypertrophy, apoptosis and inflammation through activation of various signaling cascades redox sensitive transcriptional factors. Overload of reactive oxygen species (ROS), particularly free radicals oxidizes various molecules. Lipid peroxidation and protein oxidation induces over-expression of redox genes, intracellular calcium overload and DNA fragmentation results in the damage of vascular smooth muscle cells (VSMC), endothelial cells and myocardial cells. This mechanism induces the development of atherosclerosis and cardiovascular disease.[6] ***Correlation between HbA1c and oxidative stress***

The present study was found significant positive correlation between glycosylated hemoglobin (HbA1c) and oxidative stress (Table 2). Poor glycaemic control was associated with hypoglycaemia due to excess of glucose converted to glycated hemoglobin (HbA1c). This glycaemia may play an important role in the pathogenesis and development of cardiovascular events. In hyperglycaemic condition, glucose gets oxidized and increases the production of free radicals simultaneously declines antioxidants. This oxidative stress plays a predominant role in the initiation and progression of atherosclerosis and then to cardiovascular disease. Since, both HbA1c and oxidative were increased in poor glycaemic status of type 2 diabetic patients. It shows a strong association of poor glycaemic control with HbA1c and oxidative stress. HbA1c may be a significant factor to predict risk of cardiovascular disease in type 2 diabetes mellitus.

CONCLUSION

Increased level of HbA1c and oxidative stress was found in patients with type 2 diabetes mellitus. We also have found positive association between HbA1c and Oxidative stress. This indicates that HbA1c also plays a key role in the pathogenesis of atherosclerosis and cardiovascular disease. Regular monitoring of HbA1c and MDA along with blood sugar level may helpful to forecast the

cardiovascular events. A comparative study is required on HbA1c level and oxidative stress in type 2 diabetic patients with and without cardiovascular disease.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Kilpatrick ES, Rigby AS, Atkin SL. Mean blood glucose compared with HbA1c in the prediction of cardiovascular disease in patients with type 1 diabetes. Diabetologia. 2008;51:365-71.
2. Muntner P, Wildman RP, Reynolds K, Desalvo KB, Chen J, Fonseca V. Relationship between HbA1c level and peripheral arterial disease. Diabetes care. 2005;28(8):1981-7.
3. Zoungas S, Chalmers J, Ninomiya T, Li Q, Cooper ME, Colagiuri S, et al. Association of HbA1c levels with vascular complications and death in patients with type 2 diabetes: evidence of glycaemic thresholds. Diabetologia. 2012;55:636-43.
4. Lakshmi SVV, Padmaja G, Kuppusamy P, Kutala VK. Oxidative stress in cardiovascular disease. Indian J Biochem Biophy. 2009;46:421-40.
5. Dominiczaks AF. Oxidative stress and cardiovascular disease. Endocrine Abstracts. 2005;9:S23.
6. Higashi Y, Noma K, Yoshizumi M, Kihara Y. Review Endothelial function and oxidative stress in cardiovascular disease. Circulation J. 2009;73:411-8.
7. Lipska KJ, Warton EM, Huang ES, Moffet HH, Inzucchi SE, Krumholz HM, et al. HbA1c and risk of severe hypoglycemia in type 2 diabetes. Diabetes Care. 2013;36(11):3535-42.
8. Ganz ML, Wintfeld N, Li Q, Alas V, Langer J, Hammer M. The association of body mass index with the risk of type 2 diabetes: a case-control study nested in an electronic health record system in the United States. Diabetol Metabol Syndrome 2014;6:50.
9. Hollander P. Anti-diabetes and anti-obesity medication: Effects on weight in people with diabetes. Diabetes Spectrum 2007;20(3):159-65.
10. Khan R. Weight gain and Insulin therapy. Br J Diabetes Vascular Disease. 2004;4:264-7.
11. Dokken BB, Tsao TS. The physiology of body weight regulation:Are we too efficient for our own good? Diabetes Spectrum. 2007;20(3):166-70.
12. Day C, Bailey CJ. Obesity in the pathogenesis of type 2 diabetes. Br J Diabetes Vascular Disease. 2011;11:55-61.
13. Jee SH, Sull JW, Park J, Lee SY, Ohrr H, Guallar E, et al. Body mass index and mortality in Korean men and women. N Engl J Med. 2006;355:779-87.
14. Chen Z, Yang G, Offer A, Zhou M, Smith M, Peto R, et al. Body mass index and mortality in China:a 15-year prospective study of 220000 men. Int J Epidemiol. 2012;41:472-81.
15. Ni Mhurchu C, Rodgers A, Pan WH, Gu DF, Woodward M; Asia Pacific Cohort Studies Collaboration. Body mass index and cardiovascular disease in the Asia-Pacific Region:an overview of cohorts involving 310000 participants. Int J Epidemiol. 2004;33:751-8.
16. Song SH, Hardisty CA. Type 2 Diabetes mellitus:a high risk condition for cardiovascular disease irrespective of the different degrees of obesity. Q J Med. 2008;101:875-9.
17. Klein R, Klein BEK, Moss SE. Is obesity related to microvascular and macrovascular complications in diabetes? The Wisconsin epidemiological study of diabetic retinopathy. Arch Intern Med. 1997;157:650-6.
18. Ong CR, Molyneaux LM, Constantino MI, Twigg SM, Yue DK. Long term efficacy of metformin therapy in nonobese individuals with type 2 diabetes. Diabetes care. 2006;29:2361-4.
19. Turner RC, Millns H, Holman RR. Coronary heart disease and risk factors in NIDDM – experience from the United Kingdom Prospective Diabetes Study. Diabetol. 1997;40:121-2.
20. Nathan DM, Turgeon H, Regan S. Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia. 2007;50:2239-44.
21. Little RR, Roberts WL. A Review of variant hemoglobins interfering with hemoglobin A1c measurement. J Diabetes Sci Tech. 2009;3.3:446-51.
22. Pasupathi P, Manivannan P, Uma M, Deepa M. Glycated haemoglobin (HbA1c) as a stable indicator of type 2 diabetes. Int J Pharm Biomed Res. 2010;1.2:53-6.
23. Miller ME, Bonds DE, Gerstein HC, Seaquist ER, Bergenstal RM, Calles-Escandon J, et al. The effect of baseline characteristics, glycaemia treatment approach and glycated haemoglobin concentration on the risk of severe hypoglycaemia:post hoc epidemiological analysis of the ACCORD study. BMJ. 2010;340:b5444.
24. Hong LF, Li XL, Guo YL, Luo SH, Zhu CG, Qing P, et al. Glycosylated hemoglobin A1c as a marker predicting the severity of coronary artery disease and early outcome in patients with stable angina. Lipids Health Disease. 2014;13:89.
25. Ravipati G, Aronow WS, Ahn C, Kumbar S, Saulle LN, et al. Association of hemoglobin A(1c) level with the severity of coronary artery disease in patients with diabetes mellitus. Am J Cardiol. 2006;97:968-9.
26. Saleem T, Mohammad KH, Abdel-Fattah MM, Abbasi AH. Association of glycosylated hemoglobin level and diabetes mellitus duration with the severity of coronary artery disease. Diab Vasc Dis Res. 2008;5:184-9.

27. Ikeda F, Doi Y, Ninomiya T, Hirakawa Y, Mukai N, Hata J, et al. Haemoglobin A1c even within non-diabetic level is a predictor of cardiovascular disease in general Japanese population: The Hisayama Study. Cardiovascular Diabetol. 2013;12:164.
28. Reaven PD, Moritz TE, Schwenke DC, Anderson RJ, Criqui M, Detrano R, et al. Intensive glucose lowering therapy reduces cardiovascular disease events in veterans affairs diabetes trial participants with lower calcified coronary atherosclerosis. Diabetes. 2009;58(11):2642-8.
29. Bots SH, Graaf YV, Nathoe HMW, Borst GJ, Kappelle JL, Visseren FLJ, et al. The influence of baseline risk on the relation between HbA1c and risk for new cardiovascular events and mortality in patients with type 2 diabetes and symptomatic cardiovascular disease. Cardiovascular Diabetol. 2016;15:101.
30. Selvin E, Coresh J, Golden SH, Boland LL, Brancati FL, Steffes MW, et al. Glycemic control, atherosclerosis and risk factors for cardiovascular disease in individuals with diabetes. Diabetes Care. 2005;28:1965-73.
31. Sasso FC, Carbonara O, Nasti R, Campana B, Marfella R, Torella M, et al. Glucose metabolism and coronary heart disease in patients with normal glucose tolerance. JAMA. 2004;291:1857-63.
32. Adams RJ, Appleton SL, Hill CL, Wilson DH, Taylor AW, Chittleborough CR, et al. Independent association of HbA1c and incident cardiovascular disease in people without diabetes. Obesity 2009;17:559-63.
33. Pasaoglu H, Sancak B, Bukan N. Lipid peroxidation and resistance to oxidation in patients with type 2 diabetes mellitus. Tohoku J Exp Med. 2004;203:211-8.
34. Kolhe SM, Khanwelkar CC. Oxidative stress, antioxidants and metformin in type 2 diabetes mellitus. J Med Educ Res. 2013;3(2):25-32.
35. Alam R, Khan S, Salman KA. MDA and antioxidant status in type 2 diabetes mellitus. NJIRM. 2013;4.6:75-8.
36. Wolff SP, Dean RT. Glucose auto-oxidation and protein modification. The potential role of 'auto-oxidative glycosylation' in diabetes. Biochem J. 1987;245:243-50.
37. Jain SK, Lim G. Pyridoxine and pyridoxamine inhibits superoxide radicals and prevents lipid peroxidation, protein, glycosylation and Na+P ATPase activity reduction in high glucose treated human erythrocytes. Free Radic Bio Med. 2001;30:232-7.
38. Noberasco G, Odetti P, Boeri D, Maiello M, Adezati L. Malondialdehyde (MDA) level in diabetic subjects. Relationship with blood glucose and glycosylated hemoglobin. Biomed Pharmacother. 1991;45(1-5):193-6.
39. Marchi ED, Baldassari F, Bononi A, Wieckowski MR, Pinton P. Review Oxidative stress in cardiovascular disease and Obesity:Role of p66Shc and protein kinase C. Oxid Med Cell Longev. 2013;2013:564961.

Cite this article as: Palem SP. HbA1c is a risk factor for cardiovascular disease in association with oxidative stress in patients with type 2 diabetes mellitus. Int J Res Med Sci 2017;5:3114-9.