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Original Article

Association of Asymmetrical Dimethyl Arginine with Diabetes Mellitus and Coronary Artery Disease

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ABSTRACT

Background: The individuals with Diabetes Mellitus (DM) have a large incidence of coronary artery diseases.

Objective: To compare the association of serum Asymmetrical dimethyl arginine (ADMA) level in diabetic patients with and without coronary artery disease. Serum ADMA acts as an endogenous nitric oxide synthase (NOS) inhibitor by competing with arginine for the active site. It is increased in the plasma of humans in different abnormal conditions like cardiac diseases, diabetes, atherosclerosis, hypercholesterolemia, hypertension and chronic renal failure

Methods: This analytical cross sectional study was conducted in the medical departments of Khyber teaching hospital & Hayatabad medical complex from October 2010 to October 2011. Both males & females' patients of age range of 35-65 years were included in the study. Of 210 participants 70 were included in three groups, A: normal participants, B: Type 2diabetic patients & C: diabetic patients with coronary artery diasease(CAD). Biochemical analysis was done on blood sample of these participants for ADMA, lipid profile, HbA1C & fasting blood sugar.

Results: ADMA was found to be raised in both the diabetics groups with & without CAD as compared to the normal participants (03.9±1.5µmol/L vs. 02.0±0.6 and 0.6±0.2 µmol/L) p <0.0001. Amongst the diabetic groups ADMA was higher in the diabetics with CAD as compared to diabetics without CAD. Similarly lipid profile values of LDL, Total cholesterol, triglycerides were higher in the two groups of diabetics with & without CAD as compared to the normal individuals.

Conclusion: The diabetic patients with coronary artery disease had significantly higher serum ADMA concentration than simple diabetics and normal healthy subjects.

Keywords: Type 2 diabetes mellitus (T2DM), Coronary artery disease (CAD), Glycosylated hemoglobin (HbA1c), Asymmetrical dimethyl arginine (ADMA).

Introduction

Research has proven that the endothelium plays a vital role in maintenance of vascular composition as well as its tone and is regulated by many of factors [1]. One of the major regularity mediators (endothelium-derived vasoactive mediators) is nitric oxide (NO). Owing to its cell regulatory function, nitric oxide (NO) is engaged in the pathogenesis of many degenerative disorders including atherosclerotic heart disease and type II diabetes [2]. Serum ADMA acts as an endogenous nitric oxide synthase (NOS) inhibitor by competing with arginine for the active site. This leads to reduce NO generation [3].

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ADMA is increased in the plasma of humans in different abnormal conditions like cardiac diseases, diabetes, atherosclerosis, hypercholesterolemia, hypertension and chronic renal failure [4]. Increased ADMA levels are associated with reduced NO synthesis as assessed by impaired endothelium-dependent vasodilatation [5].

Diabetes mellitus (DM) is the only effective disease classified as an epidemic [6]. The high incidence of DM is partly due to the genetic predisposition and partly due to the lifestyle of individual. Hence genetically determined susceptibility and lifestyle are the two important fascinating factors linked to the increase in the incidence of diabetes mellitus [7].One of the causes of increased ADMA concentration is hyperglycemia, a salient feature of DM. The mortality and morbidity due to chronology of types of diabetes results from complications of different organopathy i.e. diabetic neuropathy, retinopathy and nephropathy etc. such complications are a consequences of metabolic

disorder which leads to hyperglycemia [8,9]. The enzyme [dimethylarginine dimethyl amino hydrolases (DDAH)] is responsible for most of the degradation of ADMA, is impaired by hyperglycemia leading to increased concentration of ADMA[10].

The individuals with DM have a large incidence of coronary artery diseases (CADs) as well as peripheral vascular diseases partly because of DM accelerated atherogenesis [11]. In spite of the availability of latest and new therapies, still it has not been probable to efficiently decrease the high burden of cardio-vascular morbidity and mortality connected with diabetes mellitus[12].

World Health Organization has stated type 2 diabetes as a global health burden [13] . Endothelial dysfunction is an early indicator of coronary artery diseases (CADs) as well as peripheral vascular diseases [14]. One of the complex mechanisms by which hyperglycemia causes endothelial dysfunction & thus CAD is by increasing the breakdown of tissues leading to elevated levels of ADMA, which decreases the concentration of NO by inhibiting NO synthase [15]. Therefore our study aimed to look for any association of serum ADMA in type 2 diabetics in causation & progression of CAD while keeping the standard relevant clinical & laboratory data.

Materials & Methods

After being approved by the Ethical committee of Khyber medical college Peshawar (06/Bio/KMC), a cross sectional study (registration no.94-AMC-2475) was undertaken in the Cardiology departments of Khyber teaching hospital & Hayatabad medical complex from October 2010 to October 2011.A total of 210 participants were recruited in the study, of which 70 participants were in each of the three groups i.e; Group A: healthy individuals, group B-patients with T2DM for the last three years and group C: type 2 diabetic patients with CAD in the last seven days. Both males & females of age between 35-60 years fulfilling the inclusion criteria were included in the study. Those patients who were on lipid lowering drugs or renin angiotensin inhibitors as well as having renal ,liver , thyroid or inflammatory diseases were excluded from the study. A written informed consent was obtained from the participants before enrolling them in this study. A 5ml fasting blood sample obtained &2ml of this was put into EDTA tube for HbA1c estimation & the rest into eppendorf tubes after centrifugation at 3000rpm for 15 minutes for ADMA, Lipid profile &fasting blood sugar estimation.

Blood glucose (fasting), total cholesterol level, total triglyceride and HDL-cholesterol were analyzed by the enzymatic colorimetric method. LDL was calculated using Friedwald formula [16] and HbA1c level was estimated by chromatographic colorimetric method. Serum ADMA level was measured using commercially available enzyme-linked immunosorbent assay (ELISA) kit. All laboratory investigations were done at Research laboratory Biochemistry department Khyber medical college, Peshawar Khyber Pakhtunkhwa.

SPSS version 15 was used to record data. Descriptive statistics was used to calculate frequencies, percentages and graphs, for categorical variables while means ± SD was calculated for continuous variables like age, HbA1c and ADMA etc. Inferential statistic was also used to observe mean scores and was compared using Pearson's correlation. A p-value of < 0.05 was considered as statistically significant.

Results

The age of the participants ranged from 35 to 65 years with mean of 53.73 ± 6.436 SD and median 55 years indicating that 50% of the patients were from above 55 years of age group, while a similar proportion was from below 55 years age group. Further detailed categorization of age indicates that 97 (46.2%) subjects were from 46 to 55 years of age group followed by 85 (40.5%) in 56 to 65 years, while those aged <45 years of age accounted for the smallest proportion 28 (13.3%), (figure 1).

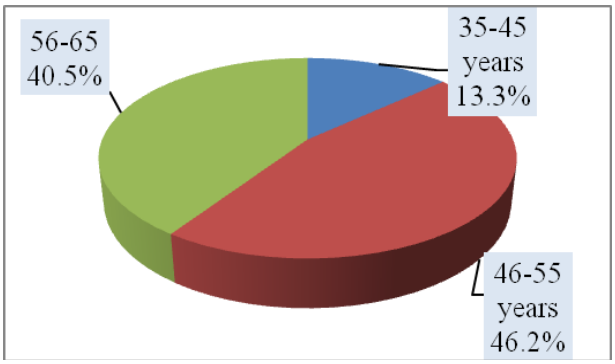

Figure 1: Age categorization of the participants

Table 1 shows association between serum ADMA level with hyperglycemia and lipid profile in normal control group. A highly significant positive correlation was observed between serum ADMA with total cholesterol (r =0.523 , p=0.0001) and triglyceride level (r = 0.439, p = 0.0001),whereas a significant correlation exists between ADMA with

HbA1c (r = 0.312 ,p = 0.009) , LDL-C level (r = 0.360 ,p = 0.002) and FBS (r =0.204 ,p =0.009). A nonsignificant correlation was observed between ADMA and HDL-C (r = 0.090 , p = 0.458).

Table 1: Correlation of asymmetric dimethylarginine (ADMA) with different parameters in Normal Control group

Biomedical Indicators	Group A (Controls)	r- value	P- value
Age in Year	50.8±8.0	0.060	0.621
Systolic Blood Pressure	119.0±10.8	0.178	0.141
Diastolic Blood Pressure	81.1±9.1	0.118	0.120
Body Mass Index	30.9±3.0	0.344	0.004**
Fasting Blood Sugar	99.7±18.4	0.204	0.090
Glycosylated hemoglobin	04.9±1.48	0.312	0.009
Cholesterol level	154.3±22.8	0.523	0.0001***
Triglyceride level	118.0±55.0	0.439	0.0001***
Low-density lipoprotein (LDL-C)	141.8±36.3	0.360	0.002**
High-density lipoprotein (HDL-C)	45.8±10.7	0.090	0.458

* Correlation is significant at the 0.05 level (2-tailed).

Table2 shows association of serum ADMA with hyperglycemia and lipid profile in patients with type2 diabetes mellitus. A significant strong positive correlation was observed between serum ADMA level and FBS(r=0.743 ,p=0.001) ,HbA1c (r=0.682 ,p=0.001) and LDL-C (r=0.508 ,p=0.001) respectively .A significant correlation exists between serum ADMA and HDL-C (r=0.288 , p=0.016), whereas a nonsignificant weak correlation was found between ADMA and total cholesterol level (r =0.204 ,p =0.090).

Table 2: Correlation of asymmetric dimethylarginine (ADMA) with different parameters in diabetic patients

Biomedical Indicators	Group B (Diabetics)	r- value	P-value
Age in Year	54.4±5.2	0.143	0.238
Systolic Blood Pressure	154.4±21.6	0.051	0.674
Diastolic Blood Pressure	92.2±10.2	0.055	0.654
Body Mass Index	28.4±3.0	0.073	0.549
Fasting Blood Sugar	170.5±60.7	0.743	0.001**
Glycosylated hemoglobin	07.4±2.8	0.682	0.001**
Cholesterol level	278.5±124.0	0.204	0.090
Triglyceride level	276.2±154.0	0.084	0.490
Low-density lipoprotein (LDL-C)	167.6±38.7	0.508	0.001**
High-density lipoprotein (HDL-C)	38.5±9.3	0.288	0.016*

Table 3 shows association of serum ADMA with hyperglycemia and lipid profile in diabetic patients with CAD . A significant and strong positive correlation was observed between serum ADMA level and FBS (r =0.633 , p= 0.001) ,HbA1c (r=0.545 , p=0.001), triglyceride level (r=0.496 , p=0.001) and LDL –C (r=0.491 ,p=0.001) ,whereas a weak significant correlation was found between ADMA and total cholesterol (r=0.392 , p=0.010).

Figure 2 shows the comparison of serum ADMA level amongst the three groups. As shown the serum ADMA level in group C (diabetics with CAD) is much higher as compared to the diabetics without CAD & normal participants. As compared to the normal value of serum ADMA i.e; 0.4 µmol/L, the ADMA levels in group B is also much higher..

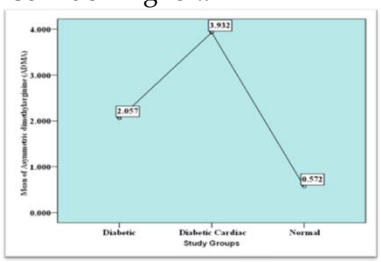

Figure 2: Comparison of serum ADMA concentration of the study groups

Table 3 Correlation of asymmetric dimethylarginine (ADMA) with different parameters in diabetic patients with CAD

Biomedical Indicators	Group C (Diabetic with CAD)	r- value	P- value
Age in Year	56.0±4.41	0.178	0.140
Systolic Blood Pressure	151.6±25.7	0.672	0.001**
Diastolic Blood Pressure	92.6±2.0	0.785	0.001**
Body Mass Index	34.9±31.0	0.383	0.001**
Fasting Blood Sugar	196.4±98.5	0.633	0.001**
Glycosylated hemoglobin	14.9±29.0	0.545	0.001**
Cholesterol level	307.4±160.1	0.392	0.010*
Triglyceride level	284.3±150.2	0.496	0.001**
Low-density lipoprotein (LDL-C)	193.8±50.7	0.491	0.001**

* Correlation is significant at the 0.05 level (2-tailed).

Discussion

It is broadly documented that patients who suffers with diabetes mellitus are at high risk of vascular complications, therefore DM is thought to be a major risk factor for developing cardio- cardiovascular diseases[17,18]. Mortality due to myocardial infarction or ischemic heart disease account for 2 to 6 times greater and the risk of stroke is 2 to 3 times greater in patients with type 2 diabetes than normal population [19,20]. Cardiovascular problems are the main cause of mortality and morbidity for the 285 million individuals internationally with type 2 diabetes mellitus [21].

Individuals with DM or with insulin resistance have impaired endothelium dependent vasodilation and other micro and macro vascular complications as compared to the normal individuals suggesting that impaired glucose tolerance and DM results in an impaired nitric oxide formation or its bioavailability in the endothelium [22,23].

Our study showed that serum ADMA level was high (03.9±1.5 µmol/L) among diabetic patients with CAD as compared to diabetic patients without CAD (02.0±0.6 µmol/L) and normal (0.6±0.2 µmol/L) subjects, this proved that in T2DM there is endothelial dysfunction caused by vascular damage which is aggravated by inflammatory markers as well as high levels of serum ADMA, all leading to CAD. Similar findings were reported in a study done by Cristina Bergmann *et al*. who evaluated the association between high plasma ADMA levels in patients with hypertension and the presence of cardiovascular risk factors and the deve

lopment f type2 diabetes mellitus and cardiovascular outcomes including death [24]. Their study confirms the association of high plasma ADMA levels and the presence of cardiovascular risk factors in patients with hypertension and suggests positive predictive value of high plasma ADMA levels for cardiovascular death in patients with hypertension and also for the development of type2 DM in the sub group of patients with hyper tension free from metabolic abnormalities.

In an another study done by Mustafa Celik *et al.* showed that there is a relationship between ADMA and macro vascular disease in type 2 diabetes, the ADMA level was found to be higher in diabetic patients with macro vascular complications compared to diabetic patients without complications. When all the diabetics were compared to the control group, ADMA levels in the diabetics were significantly increased ($P < 0.001$). It was shown that ADMA tends to increase in DM, which is a main cause of atherosclerosis [25].

We found a positive correlation of serum ADMA concentration with fasting blood sugar level (r = 0.366). These findings are in accordance to a study by BS Dayir *et al.*, who demonstrated that elevated glucose levels are capable of inhibiting DDAH activity in cultured endothelial cells [26]. Clinical investigations in patients also indicate that ADMA is directly related to blood glucose levels as shown by Takaya J *et al.* and Tariq K *et al*. [27].

In our study a significant positive correlation between serum ADMA level and HbA1c was demonstrated among normal healthy individuals (r = 0.312, p = 0.009) , patients with type 2 diabetes mellitus (r =0.682 , p = 0.001) type 2 diabetic with CAD, (r = 0.545, p = 0.001).In contrast to the work done by Karakoc *et al.,* there was no significant correlation between plasma ADMA level and HbA1c

concentration [28] . While Devangelio et al (2007) conducted a study showing that ADMA level was significantly higher in diabetic patients and HbA1c and ADMA were directly correlated [29].

In the present study the serum HDL levels were low (38.5±9.3 mg/ dl) among patients with type 2 diabetes mellitus as compared to diabetics with coronary artery disease (32±07mg/dl) and normal healthy subjects (45.89±10.1 mg/dl), a strong inverse relationship between HDL and ADMA in myocardial infarction patients, suggesting a functional interaction between HDL and endothelium was also shown in a study Lorin J by *et al*. In their study, the median ADMA levels were markedly higher in the low HDL group (0.69 vs. 0.50 mmole/L, p,0.001)(30). Sibel *et al*. showed the effect of statin therapy on serum ADMA level , they found that increase LDL-cholesterol levels were associated with increase serum ADMA level and this elevated ADMA concentration is associated with increased risk of cardiovascular disease [8]. Our study also showed increase serum level of LDL in diabetic patients with CAD (193.8 ± 50.7) and in type 2 diabetic patients (167.6 ± 38.7) as compared to the normal control group (141.8 ± 36).

In another study done by Boger *et al*. , it was found that plasma ADMA levels are doubled in hyper cholestrolemic humans in association with evidence of reduced NO synthesis [31] ,similar to this study , a significant linear correlation was seen of ADMA level with cholesterol level and triglyceride level (r = 0.392, p = 0.01; and r = 0.496, p = 0.001 respectively) in type 2 diabetic patients with CAD [31].

Increased levels of high asymmetrical di methyl arginine (ADMA) and LDL-C were seen in our study, the serum level of LDL in diabetic patients with CAD (193.8 ± 50.7) and in type 2 diabetic patients (167.6 ± 38.7) as compare to the normal control group (141.8 ± 36). Protopsaltis *et al.* also revealed elevated ADMA and LDL-C levels & suggested that these were strongly associated with increased arterial stiffness among pre-diabetic subjects [32].

Conclusion

Diabetes Mellitus has a role in increasing the ADMA levels & thus causation of CAD. They also had a deranged lipid profile with increased cholesterol, LDL and decreased HDL as compared to the diabetics and normal individuals.

Recommendations:

Further studies should be conducted on larger sample size and longer duration, to explore the role of ADMA in CAD development and progression.

Conflict of Interest: Authors declare no conflict of interest.

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References

1. Krüger-Genge A, Blocki A, Franke R-P, Jung F. Vascular endothelial cell biology: an update. Int J Mol Sci. 2019;20(18):4411.
2. Król M, Kepinska M. Human nitric oxide Synthase—Its functions, polymorphisms, and inhibitors in the context of inflammation, diabetes and cardiovascular diseases. Int J Mol Sci. 2020;22(1):56.
3. Böger R, Hannemann J. Dual role of the L-arginine–ADMA–NO pathway in systemic hypoxic vasodilation and pulmonary hypoxic vasoconstriction. Pulm Circ. 2020;10(1_suppl):23–30.
4. Gajjala PR, Sanati M, Jankowski J. Cellular and molecular mechanisms of chronic kidney disease with diabetes mellitus and cardiovascular diseases as its comorbidities. Front Immunol. 2015;6:340.
5. Cziráki A, Lenkey Z, Sulyok E, Szokodi I, Koller A. L-arginine-nitric oxide-asymmetric dimethylarginine pathway and the coronary circulation: translation of basic science results to clinical practice. Front Pharmacol. 2020;11:569914.
6. Lovic D, Piperidou A, Zografou I, Grassos H, Pittaras A, Manolis A. The growing epidemic of diabetes mellitus. Curr Vasc Pharmacol. 2020;18(2):104–9.
7. Mambiya M, Shang M, Wang Y, Li Q, Liu S, Yang L, et al. The play of genes and non-genetic factors on type 2 diabetes. Front public Heal. 2019;7:349.
8. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress—A concise review. Saudi Pharm J. 2016;24(5):547–53.
9. Chawla A, Chawla R, Jaggi S. Microvasular and macrovascular complications in diabetes mellitus: distinct or continuum? Indian J Endocrinol Metab. 2016;20(4):546.
10. Hulin J-A, Gubareva EA, Jarzebska N, Rodionov RN, Mangoni AA, Tommasi S. Inhibition of dimethylarginine dimethylaminohydrolase (DDAH) enzymes as an emerging therapeutic strategy to target angiogenesis and vasculogenic mimicry in cancer. Front Oncol. 2020;9:1455.
11. Glovaci D, Fan W, Wong ND. Epidemiology of diabetes mellitus and cardiovascular disease. Curr Cardiol Rep. 2019;21(4):1–8.
12. Srivastava RAK. Dysfunctional HDL in diabetes mellitus and its role in the pathogenesis of cardiovascular disease. Mol Cell Biochem. 2018;440(1):167–87.
13. Jaacks LM, Siegel KR, Gujral UP, Narayan KMV. Type 2 diabetes: A 21st century epidemic. Best Pract Res Clin Endocrinol Metab. 2016;30(3):331–43.

14. Medina-Leyte DJ, Zepeda-García O, Domínguez-Pérez M, González-Garrido A, Villarreal-Molina T, Jacobo-Albavera L. Endothelial dysfunction, inflammation and coronary artery disease: potential biomarkers and promising therapeutical approaches. Int J Mol Sci. 2021;22(8):3850.
15. Tain Y-L, Hsu C-N. Toxic dimethylarginines: asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA). Toxins (Basel). 2017;9(3):92.
16. Rifai N, Warnick GR, McNamara JR, Belcher JD, Grinstead GF, Frantz Jr ID. Measurement of low-density-lipoprotein cholesterol in serum: a status report. Clin Chem. 1992;38(1):150–60.
17. Papatheodorou K, Banach M, Bekiari E, Rizzo M, Edmonds M. Complications of diabetes 2017. Vol. 2018, Journal of diabetes research. Hindawi; 2018.
18. Domingueti CP, Dusse LMS, das Graças Carvalho M, de Sousa LP, Gomes KB, Fernandes AP. Diabetes mellitus: the linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. J Diabetes Complications. 2016;30(4):738–45.
19. Gierlotka M, Zdrojewski T, Wojtyniak B, Poloński L, Stokwiszewski J, Gąsior M, et al. Incidence, treatment, in-hospital mortality and one-year outcomes of acute myocardial infarction in Poland in 2009–2012—nationwide AMI-PL database. Kardiol Pol (Polish Hear Journal). 2015;73(3):142–58.
20. Rawshani A, Rawshani A, Franzén S, Sattar N, Eliasson B, Svensson A-M, et al. Risk factors, mortality, and cardiovascular outcomes in patients with type 2 diabetes. N Engl J Med. 2018;
21. Genser L, Mariolo JRC, Castagneto-Gissey L, Panagiotopoulos S, Rubino F. Obesity, type 2 diabetes, and the metabolic syndrome: pathophysiologic relationships and guidelines for surgical intervention. Surg Clin. 2016;96(4):681–701.
22. Patel TP, Rawal K, Bagchi AK, Akolkar G, Bernardes N, Dias D da S, et al. Insulin resistance: an additional risk factor in the pathogenesis of cardiovascular disease in type 2 diabetes. Heart Fail Rev. 2016;21(1):11–23.
23. Kaur R, Kaur M, Singh J. Endothelial dysfunction and platelet hyperactivity in type 2 diabetes mellitus: molecular insights and therapeutic strategies. Cardiovasc Diabetol. 2018;17(1):1–17.
24. Triches CB, Mayer S, Marie B, Quinto R, Costa M, Zanella MT. Association of endothelial dysfunction with cardiovascular risk factors and new- - onset diabetes mellitus in patients with hypertension. 2018;(November 2017):935–41.
25. Celik M, Cerrah S, Arabul M, Akalin A. Relation of Asymmetric Dimethylarginine Levels to Macrovascular Disease and Inflammation Markers in Type 2 Diabetic Patients. 2014;2014.
26. Dayir BS, Hamzah M, Khudair MSH. Serum levels of Asymmetric dimethyl arginine and Nitric oxide in patients with prediabetes and type2 diabetes mellitus. Ann Trop Med Heal. 2020;23:11–6.
27. Takaya J, Tanabe Y, Kuroyanagi Y, Kaneko K. Asymmetric dimethylarginine is negatively correlated with hyperglycemia in children. Endocr J. 2015;EJ14-0521.
28. Karakoç A, Sahin A, Polat ES, Aliyev E, Yildirim A, Bakan N, et al. Serum apelin and ADMA levels in type 2 diabetics with and without vascular complications. Diabetes Metab Syndr Clin Res Rev. 2016;10(2):S106–9.
29. Triches CB, Mayer S, Quinto BMR, Batista MC, Zanella MT. Association of endothelial dysfunction with cardiovascular risk factors and new-onset diabetes mellitus in patients with hypertension. J Clin Hypertens. 2018;20(5):935–41.
30. Lorin J, Guilland J, Korandji C, Touzery C, Bichat F, Chagnon A, et al. High Levels of Asymmetric Dimethylarginine Are Strongly Associated with Low HDL in Patients with Acute Myocardial Infarction. 2013;8(6):1–7.
31. Bo RH, Bode-bo SM, Szuba A, Tsao PS, Chan JR, Tangphao O, et al. Clinical Investigation and Reports Asymmetric Dimethylarginine (ADMA): A Novel Risk Its Role in Hypercholesterolemia. 1998;1842–7.
32. Protopsaltis I, Foussas S, Angelidi A, Gritzapis A, Sergentanis TN, Matsagos S, et al. Impact of ADMA , endothelial progenitor cells and traditional cardiovascular risk factors on pulse wave velocity among prediabetic individuals. 2012;1–9.

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C. Interpretation/ Analysis and Discussion