Original article:

Asymmetrical Dimethylarginine(ADMA), an Endothelial Dysfunctional Biomarker for Type 2 Diabetes Mellitus, the Meta Analysis

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Abstract:-

Introduction: Diabetes Mellitus is one of the diseases with many complications which requirea novel biomarker for early diagnosis and treatment. Asymmetrical dimethylarginine (ADMA)an endothelial dysfunction biomarker as proven to be a promising biomarker in type 2 diabetes mellitus (T2DM).

Method:-Data extraction was conducted independently using a standardized data extraction form. Only full length text articles were included in the study. For each included article, we extracted information on the title, authors, publication year, name of the study, sample size, number of diabetes cases and control study, mean (standard deviation) for the ADMA level, assay for measuring ADMA levels, and statistical methods used for the analysis.

Observations and results: Level of serum ADMA were highly significantly elevated in patients T2DM comparedto healthy control.(Standardmeandeference [SMD]: +0.58, CI:+0.55 to +0.61). The result of each study also showed that serum ADMA in T2DM group was higher than that in healthy control subjects. Ourmeta-analysis showed that there was highly significant positive association between ADMA and endothelial dysfunctional risk of T2DM (t: 36.25, df-1042: p=0.0000000****).

Conclusion: ADMA when used as endothelial dysfunction biomarker of T2DM, helps in early diagnosis ,effective therapeutic strategies and monitoring T2DM.

Key wards:-T2DM-type 2 diabetes mellitus,Asymmetrical dimethylarginine (ADMA) , Endothelial dysfunction.

Introduction:

Cardiovascular disease is a leading cause of mortality and morbidity for the 135 million individuals worldwide affected by type 2 diabetes mellitus (T2DM).[1–3]

Endothelial dysfunction is a common feature in diabetic patients and may contribute to cardiovascular complications. Mechanisms of diabetes-induced endothelial dysfunction include the production of prostanoid vasoconstrictors and the increased oxidative degradation of Nitric oxide (NO). Deficiency of NO increases vascular resistance and promotes atherogenesis. In addition to its increased oxidative degradation, another possible mechanism for NO deficiency and cardiovascular morbidity is reduced NO synthesis caused by ADMA.[4]ADMA is

an endogenous competitive inhibitor of NO synthase (NOS). This modified amino acid is derived from proteins that have been post-translationally methylated and subsequently hydrolyzed. ADMA is in part cleared by renal excretion. Reduced clearance of ADMA in renal failure is associated with endothelial vasodilator dysfunction, reversible by administration of L-arginine or by dialysis, which removes plasma ADMA. However, the enzyme dimethylargininedimethylaminohydrolase (DDAH) accounts for most of the clearance of ADMA. DDAH metabolizes ADMA to L-citrulline and dimethylamine. ADMA is elevated to a level that can significantly inhibit NOS activity in individuals with hypercholesterolemia, hypertension, hyperhomocyst(e)inemia, tobacco exposure, and hyperglycemia of them hyperglycemias can elevate intracellular oxidative stress through multiple mechanisms.[5]

Currently,theprevalenceoftype2diabetesin the United States and many other countries in the world has reached to the level of epidemic proportions.[6]The serum concentration of ADMA, could be used to monitor early changes in the L-arginine-NO-metabolism .Epidemiologic studies can provide insight into the potentialimportanceofthese endothelial dysfunction moleculeas determinants of the incidence of Cardiovascular riskin type 2 diabetes in human populations. In addition, these studies can identify biological markers ADMA that may be useful for the prediction of cardiovascular risk in type2diabetesandtheidentificationofhigh-risk groups.

In literature search, nometa-analysis was found evaluating the available evidence for an association between ADMA levels and endothelial dysfunctional cardiovascular riskintype 2 diabetes across different populations. Hence the current study was planned.

Aim and Objectives:-

1. To evaluate the available evidence for an association between ADMA levels and endothelial dysfunctional cardiovascular risk in type 2 diabetes across different populations.

2. The objective of our meta-analysis was to assess the consistency of the association of ADMAlevels and endothelial dysfunctional risk in type 2 diabetes in and to summarize the results of the meta-analysis.

Methods:-

Search Strategy:-

Medical Literature was searched using MEDLINE (PubMed), EMBASE, Cochrane databases and through reference list. The following medical terms were used: ADMA, endothelial dysfunctional marker, Cardiovascular disease complications, microvascular and macrovascular complications and T2DM.

To increase the sensitivity ofour search, eligible studies were cross reference dusing the Science Citation Index (SCI). We also reviewed recently presented data at national and international meetings. Additionally, we searched Internet-based sources of information (www.cardiosource.com, www.google.com, www.clinicaltrialresults.org, www.theheart.org, and www.tctmd.com).

Eligibility criteria:-

We included prospective studies of plasma ADMA concentrations in type 2 diabetes and its associated diseases. We excluded literature reviews, studies on animals or cell lines, studies of determinants of ADMA levels, studies of genetic variation in ADMA-related genes, and studies of type 1 diabetesorgestational diabetes .We also excluded studies on populations with specificdiseasesorusingspecificmedications.Fourstudieswereexcludedbecausedata was notreported separately control (healthy) and diabetes. We

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included full research article on ADMA levels in (µmol/L) andtype2diabetes.

Data Extraction:-

Data extraction was conducted independently by first 2 authors using astandardized dataextraction form. Only full length text articles were included in the study. For each included article, we extracted information on the title, authors, publication year, name of the study, sample size, number of diabetes cases and control study, mean (standard deviation) for the ADMA level, assay for measuring ADMA levels ,and statistical methods used for the analysis.

Statistical analysis:-

In the analysis, 9 selected studies have been considered on the basis of sample size, methodology, result obtained and year of publication. The analysis is done with the help of statistical package SPSS latest version24. In the study, the mean ADMA level in cases and controls were compared along with the standard deviation of both. The proportional weight of each study was given as per the sample size. The individual **independent sample "t"**test was applied to each study and 95% confidence intervals are drawn. The aggregate estimate of cases and control means and standard deviation was calculated by weighted average method, where weight for each was given as per the sample size of each study. The difference in the estimate is calculated as SMD for each study along with their 95% confidence interval for the same. Combined estimation of all 9 studies is also calculated. Individual linear graph for each study (Fig 2 & Table 2)are prepared with mean and confidence intervals. Forest plot (Fig 3 & Table 3) reflects SMD and 95% CI for individual studies as well as combined estimate.

Result:-

Literature search

Literature search using the above mentioned search criteria identified106 articles in the three databases; PubMed,EMBASE & Cochrane. Four additional articleswere identified by manually searching the reference listsand forward citations of included papers. 61 articles were discarded due to duplication. Out of the remaining 49, which were screened,9studies were excluded as full text articles were not available..

40 full text articles were assessed for eligibility. Out of these, 27 full articles excluded (16 Paper are not relevant, and 9 papers are reviews, 2 papers on type 1 diabetes[7,8]and three studies where control group was not mentioned[5,6,10].One study has expressed ADMA value in different unit.[11]This strategy is summarized in Figure 1 and a descriptionof the included ADMA level as per above criteria.

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Figer-1

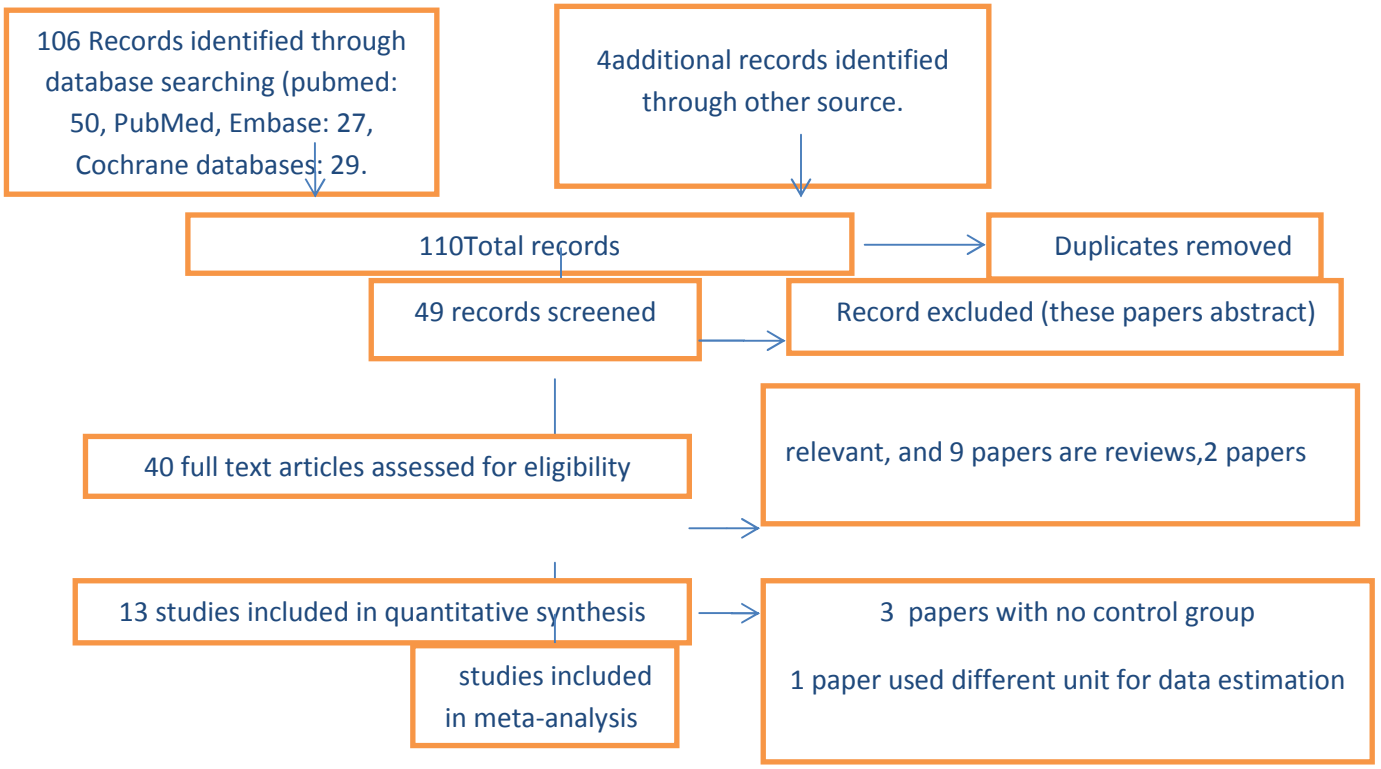
106 Records identified through database searching (pubmed: 50, PubMed, Embase: 27, Cochrane databases: 29.
4additional records identified through other source.
110Total records
Duplicates removed
49 records screened
Record excluded (these papers abstract)
40 full text articles assessed for eligibility
relevant, and 9 papers are reviews,2 papers
13 studies included in quantitative synthesis
3 papers with no control group
1 paper used different unit for data estimation
studies included in meta-analysis

Table-1 .shows the characteristics of the 9 identified studies of ADMA levels in type 2 diabetes.[12-20]Study participants were from the general populationand ADMA serum level expresses in µmol/L in both cases and control groups. The majority of the studies were published between 2012 to 2016. Only one article is published in 2002.

Table-1Description of Included studies

Together 9 studies reported 548 cases of diabetes and 496 controls. Each study showed significant higher levels of ADMA in cases as compared to controls.Independent sample t test was applied to each study and t value has been calculatedalong with degree of freedom (d.f.) and p value.

Data of all the studies was combined by using weighted average method where weight is decided as per individual

Author	**year**	**Type 2 Diabetes**		**No. case Sample**	**Control**		**No. control Sample**	**Method**
		Mean	**SD**		**Mean**	**SD**		
1)TulinBayrak[12]	2014	1.2	0.08	20	0.83	0.05	23	HPLC
2) Mohamad Mahfouz [13]	2009	1.31	0.45	20	0.41	0.092	20	ELISA
3) TaliGanz[14]	2016	0.45	0.1	105	0.42	0.09	137	HPLC
4) Mustafa Celik[15]	2016	0.45	0.21	100	0.32	0.13	31	ELISA
5) W. Xia [16]	2012	0.84	0.19	72	0.49	012	72	HPLC
6) Akira Ito [17]	2002	0.53	0.03	11	0.42	0.01	8	HPLC
7) Angela Sciacqua[18]	2012	0.7	0.2	45	0.5	0.2	30	HPLC
8) Ashok Sahu[19]	2009	0.60	0.11	100	0.530	0.101	100	ELISA
9) Kalsoom Tariq [20]	2016	03.9	1.5	75	0.6	0.2	75	ELISA

study sample size. Independent sample t test was applied to the pooled data, which was highly statistically significant (t= 36.25, df=1042 and p=0.0000000….).(Table 2& Fig 2).Standardized mean difference for individual studies and for aggregate were estimated with 95% confidence interval. This strongly supports our conclusion.(Table 3 & Fig 3)

Table-2 Application of independent sample t test to individual studies with aggregate estimate by weighted average method.

Sr.No.	Author/ Year	"t" Cal.	Degree of freedom (df)	Level of significance
1	TulinBayrak 2014[12]	18.5	41	p<0.0001
2	MohamadMahfouz 2009[13]	6.19	38	p<0.0001
3	TaliGanz2016[14]	2.5	240	p<0.05
4	Mustafa Celik 2016[15]	3.25	129	p<0.01
5	W. Xia 2012[16]	11.67	142	p<0.00001
6	Akira Ito 2002[17]	11	17	p<0.000001
7	Angela Sciacqua 2012[18]	4	73	p<0.0001
8	Ashok Sahu2009[19]	4.375	198	p<0.001
9	KalsoomTariq 2016[20]	18.86	148	p<0.0001
	CombinedEstimate	**3625**	**1042**	**P=0.0000000….**

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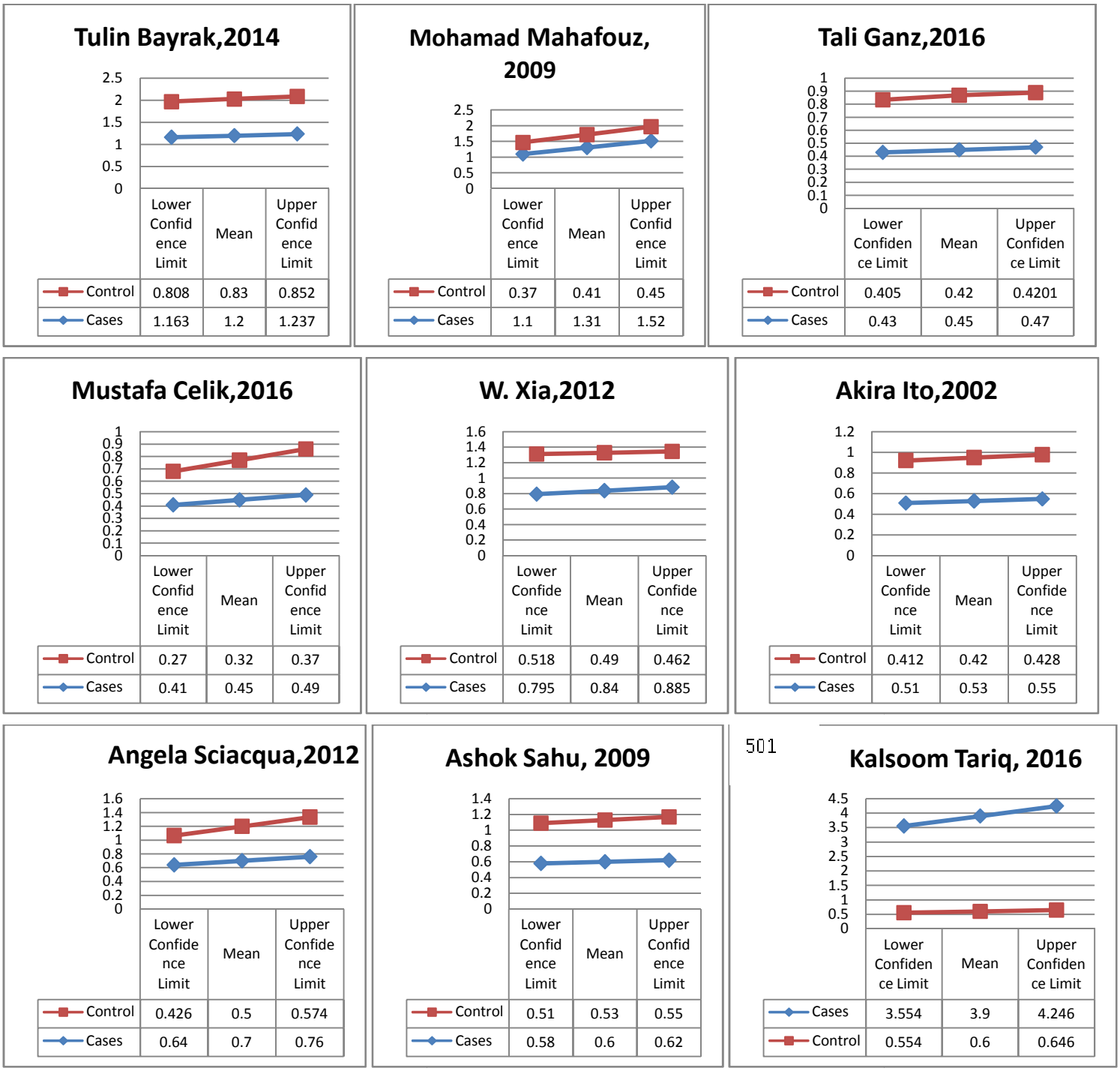
Figure 2-Linear graphs of 95% confidence intervals of ADMA levels of cases of T2DM and comparable healthy controls



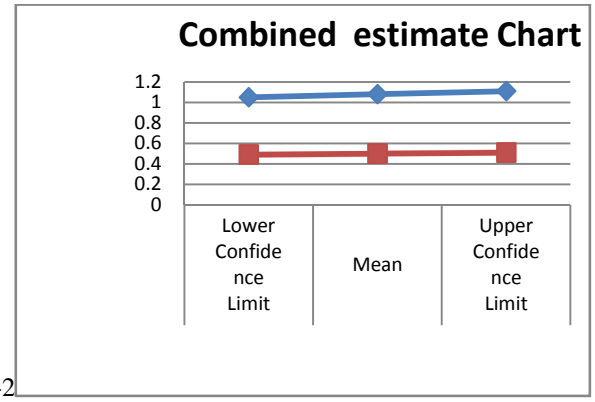
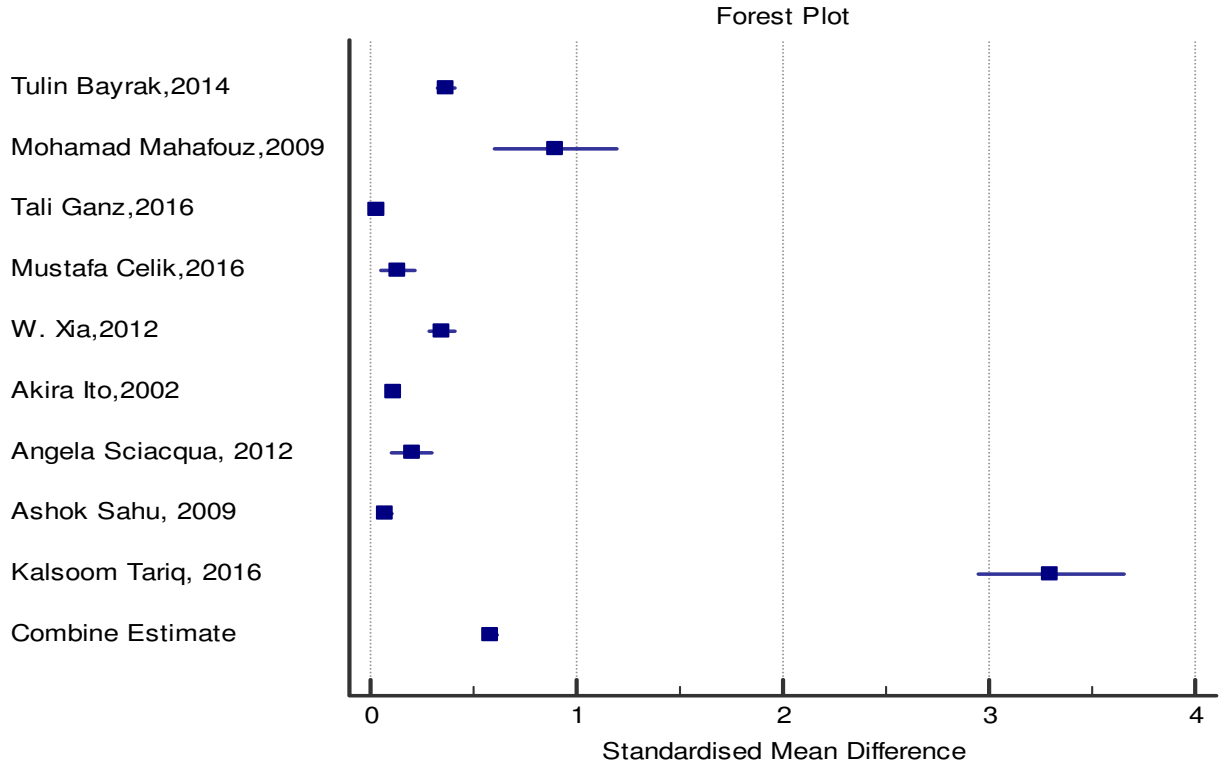


Figure-2

Forest plot for ADMA shows a significantly increased ADMA levels in T2DM cases compared to controls, the overallstandard mean deference (SMD) was +0.58(95%CI: +0.55 to +0.61) (p=0.0000000**).** (Fig3&Table3)

Figer-3 Forest plot showing the levels of ADMA in T2DM and healthy control individuals and combined study.



(SMD): +0.58 (CI: +0.55 to +0.61) (p=0.0000000…..)

Table-3 Levels of ADMA in T2DM cases and healthy control individuals and combined estimate.

Sr.No.	Study	SMD (95% CI)	Weight %
1	TulinBayrak 2014[12]	+0.37(+0.33 to 0.41)	4.12
2	MohamadMahfouz 2009[13]	+0.9(+0.605 to 1.195)	3.83
3	TaliGanz2016[14]	+0.03(+0.006 to +0.054)	23.18
4	Mustafa Celik 2016[15]	+0.13(+0.05 to +0.21)	12.55
5	W. Xia 2012[16]	+0.35 (+0.29 to +0.41)	13.79
6	Akira Ito 002[17]	+0.11 (+0.09 to +0.13)	1.82
7	Angela Sciacqua 2012[18]	+0.2 (+0.1 to +0.3)	7.18
8	Ashok Sahu2009[19]	+0.07 (+0.04 to +0.1)	19.16
9	Kalsoom Tariq 2016[20]	+3.3 (+2.95 to +3.65)	14.37
10	**Combined estimate**	**0.58 (+0.55 to +0.61)**	**100**

Discussion:-

To our knowledge ,studies where the meta-analysis is done to assess the association between ADMA and T2DM are not commonly seen. In our meta-analysis the result showed that ADMA in the T2DM group was higher than that in healthy controls. (t=36.25, df=1042 p=0.0000000….)This also shows significant positive relationship between ADMA and risk of T2DM.

In 1992, Vallance and colleagues demonstrated that N^G, N^G-dimethyl-L-arginine (asymmetric dimethylarginine [ADMA]) is detectable in plasma and is an endogenous competitive inhibiter of NO synthase (NOS).[21]An L-arginine metabolite, ADMA, L-arginine/ADMA ratio shows NO synthesis ADMA is degraded by the enzyme dimethylargininedimethylaminohydrolase (DDAH). ADMA demolishes endothelium derived flow response substantially. Increased ADMA impairs endothelium derived NO mediated vasodilation, enhances monocyte adhesion, and causes platelet hyperaggregation.[10] In recent years, animal study have indicated that ADMA and risk of T2DM and vascular complications.[22] A observational study had shown there was a negative relation between ADMA level in T2DM and healthy control[23]

Endothelial dysfunction was indicated as a major cardiovascular risk factor in various trials. Endothelial dysfunction is defined as a decrease in bioavailability of NO which inhibits the adhesion and aggregation of platelets, vascular smooth muscle cell proliferation and low density lipoprotein (LDL) oxidation, and adhesion of monocytes and leukocytes to the endothelium. ADMA is essentially a competitive inhibitor of endothelial nitric oxide synthase (eNOS). ADMA regulates the production rate of NO. Plasma ADMA concentration has been shown to increase during the course of diseases associated with endothelial dysfunction such as diabetes mellitus, peripheral artery disease, hypertension and cardiovascular diseases.[9]

Inclusion and exclusion criteria were strictly adhered to. ADMA between the T2DM and non-diabetes mellitus group were compared. Significant positive association between ADMA and T2DM was observed.

Limitations: Very few studies fulfilling all the eligibility criteria were identified. Only single

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variable like serum ADMA was involved in the analysis. Due to non-availability of the suitable data we could not estimate Odd's Ratio/ Risk Ratio.

Conclusion:-

The current study shows that increased ADMA is the predictive bio-marker of the endothelialdysfunction and cardiovascular complications in T2DM. Further ADMA is useful for prediction of type 2 diabetes in addition to established risk factors using statistical techniques appropriate for prognostic analysis. It helps for early diagnosis and effective therapeutic strategies as well as monitoringfor T2DM.

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