Original article:

Serum 8-hydroxy deoxyguanine (8-OHdG), DNA damaging Oxidative Stress Biomarker for Type 2 Diabetes Mellitus, the Meta Analysis

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Abstract:

Diabetes Mellitus is one of the diseases with many complications which require a novel biomarker for early diagnosis and treatment. 8-hydroxy deoxyguanine (8-OHdG), DNA damaging oxidative stress 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 8-HdG level, assay for measuring 8-HdG levels, and statistical methods used for the analysis.

Observations and results: Level of serum 8-HdG were highly significantly elevated in patients T2DM compared to healthy control. (Standard mean deference [SMD]) and (95% Confidence Interval [CI]) +1051.06 (+996.29 to +1135.83). The result of each study also showed that serum 8-HdG in T2DM group was higher than that in healthy control subjects. Our meta-analysis showed that there was highly significant positive association between 8-HdG and DNA damaging oxidative stress risk of T2DM (t-24.3, df-777: p=0.0000000****).

Conclusion: 8-HdG used as DNA damaging oxidative stress biomarker of T2DM, helps in early diagnosis, effective therapeutic strategies and monitoring T2DM.

Key wards:- T2DM-type 2 diabetes mellitus, 8-hydroxy deoxyguanine (8-HdG).

Introduction:-

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and insulin action or both [1]. In type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response.

Oxidative stress due to overproduction of reactive oxygen species (ROS) has important role in prevention, initiation, and progression of chronic diseases from early childhood [2] . Reactive properties of ROS cause oxidative damage to lipids, deoxyribonucleic acid (DNA), and proteins. Recent evidence suggests that ROS could have a role in the development of hypertension, dyslipidaemia, diabetes mellitus, and atherosclerosis [3]. Oxidative stress status in diabetes could be clearly demonstrated by the increase of some specific biomarkers such as lipid hydro peroxides, DNA adducts and protein carbonyls [4].

ROS can interact with DNA to produce damage including single- and double-stranded DNA breaks, deletions and nucleoside modifications. Among purine and pyrimidine base, guanine is more susceptible to oxidation. The hydroxyl radical can attack to C-8 position guanine and generate an oxidation product, 8-hydroxy deoxyguanine (8-OHdG). 8-OHdG, the oxidized form of the nucleoside 2'-deoxyguanosine present in DNA, is one of the most reliable and abundant markers of DNA damage because it reflects generalized cellular oxidative stress; it may be a risk factor for cancer, atherosclerosis and DM [3,5].

Currently, the prevalence of type 2 diabetes in the United States and many other countries in the world has reached to the level of epidemic proportions [6]. The serum concentration of 8-HgG, could be used to monitor early changes in the DNA damage. Epidemiologic studies can provide insight into the potential importance of these Oxidative stress as determinants of the incidence of Cardiovascular risk in type 2 diabetes in human populations. In addition, these studies can identify biological markers 8-HdG that may be useful for type 2 diabetes and the identification of high-risk groups.

In literature search, no meta-analysis was found evaluating the available evidence for an association between 8-HdG levels and oxidative stress in type 2 diabetes across different populations. Hence the current study was planned.

Aim and Objectives:-

1. To evaluate the available evidence for an association between 8-HdG levels and oxidative stress in type 2 diabetes across different populations.
2. The objective of our meta-analysis was to assess the consistency of the association 8-HdG levels and oxidative stress in type 2 diabetes 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: serum 8-HdG, oxidative stress marker, cardiovascular disease complications, microvascular and macrovascular complications and T2DM.To increase the sensitivity of our search; eligible studies were cross referenced using the Science Citation Index (SCI).

We also reviewed recently presented data at national and international meetings. Additionally, we searched Internet-based sources of information

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(www.cardiosource.com, www.google.com, www.clinicaltrialresults.org, www.theheart.org, and www.tctmd.com).

Eligibility criteria:-

We included prospective studies of Serum: 8-HdG concentrations in type 2 diabetes and its associated diseases. We excluded literature reviews, studies on studies on animals or cell lines, studies of determinants of 8-HdG levels, studies of genetic variation in -related: 8-HdG genes, and studies of type 1 diabetes or gestational diabetes. We also excluded studies on populations with specific diseases or using specific medications. We also excluded studies on urine 8-HdG levels in T2DM. Two studies were excluded because data was not reported separately control (healthy) and diabetes. We included full research article on 8-HdG levels in (pg/ml) and type 2 diabetes and two full articles with different unit 8-HdG levels was converted in to Pg/ml and T2DM also involved in present meta-analysis [16,17].

Data Extraction:-

Data extraction was conducted independently by first 2 authors 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 8-HdG level, assay for measuring levels, and 8-HdG statistical methods used for the analysis.

Statistical analysis:-

In the analysis, 7 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 version 24. In the study, the mean 8-HdG 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 7 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 identified 102 articles in the three databases; PubMed, EMBASE & Cochrane. Two additional articles were identified by manually searching the reference lists and forward citations of included papers. 60 articles were discarded due to duplication. Out of the remaining 44, which were screened, 5 studies were excluded as full text articles were not available. 39 full text articles were assessed for eligibility. Out of these, 26 full articles excluded (17 Paper are not relevant, and 8 papers are reviews, one papers on prediabetes[7] and one study where 8-HdG mean and SD values was not mentioned in this study[8].

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Five studies on urinary 8-HdG levels in T2DM [9-13]. This strategy is summarized in Figure 1 and a description of the included 8-HdG level as per above criteria.

Figer-1

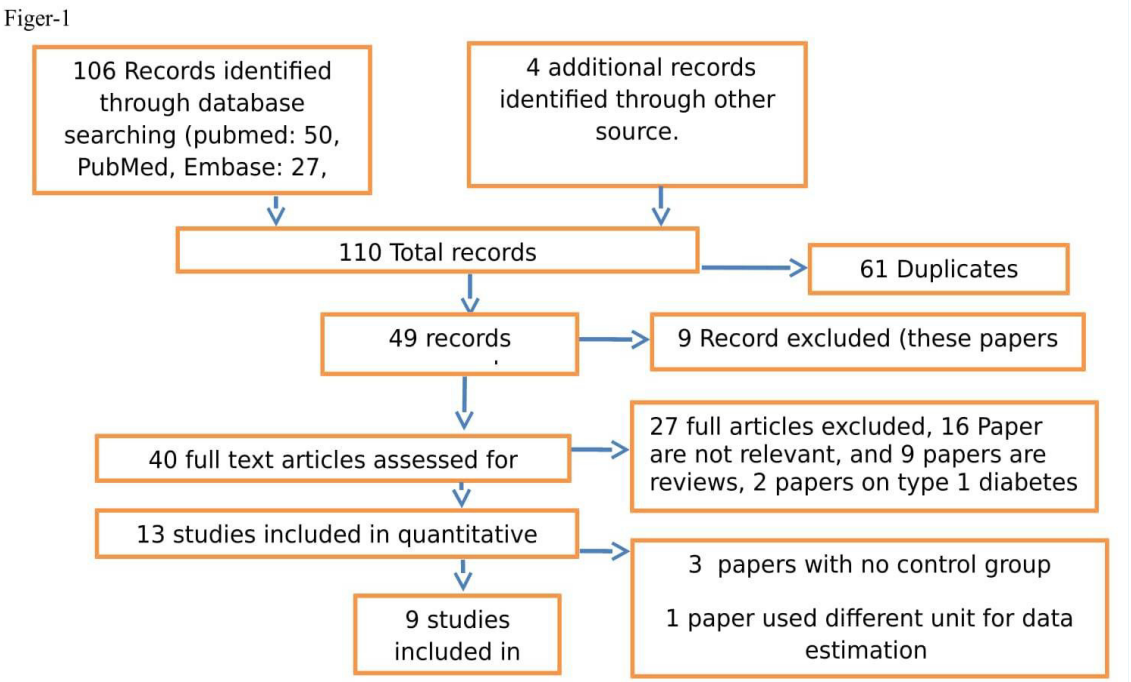

Table-1 .shows the characteristics of the 7 identified studies of 8-HdG levels in type 2 diabetes.[14-20] Study participants were from the general population and8-HdGserum level expresses in pm/ml in both cases and control groups. The majority of the studies were published between 2010 to 2016

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Table-1 Description of Included studies

Author	Year	Control		No. control Sample	Type 2 diabetes		No. cases Sample	Method	Unit
		Mean	SD		Mean	SD			
1.Hayder A. Aubaidy [14]	2010	177.8	91.1	98	1926.6	1177	35	ELISA	Pg/ml
2. Hayder A. Aubaidy [15]	2011	210.4	166	119	1979.6	1209	35	ELISA	Pg/ml
3.Omur Tabak [16]	2011	1800	540	19	2240	970	69	ELISA	Pg/ml
4.Jion Sun [17]	2015	240	140	65	720	410	28	ELISA	Pg/ml
5.Hyder A Aubaidy [18]	2011	240.9	103	9	600.4	214.4	72	ELISA	Pg/ml
6.Mohammed H.Mukhtar [19]	2016	110.2	31.46	80	178.35	26.23	50	ELISA	Pg/ml
7.Wesam Ahmed Nasif [20]	2016	84.87	16.17	50	159.38	15.40	50	ELISA	Pg/ml

Together 9 studies reported 440 controls and 339 cases of type 2 diabetes. Each study showed significant higher levels of serum 8-HdG in cases as compared to controls. Independent sample t test was applied to each study and t value has been calculated along with degree of freedom (df.) and p value.

Data of all the studies was combined by using weighted average method where weight is decided as per individual study sample size. Independent sample t test was applied to the pooled data, which was highly statistically significant (t = 24.3, df = 777 and p= 0.0000000….). (Table 2 & Fig 2). Standardized mean difference for individual studies and for aggregate were estimated with 95% confidence interval (CI). This strongly supports our conclusion. (Table 3 & Fig 3)

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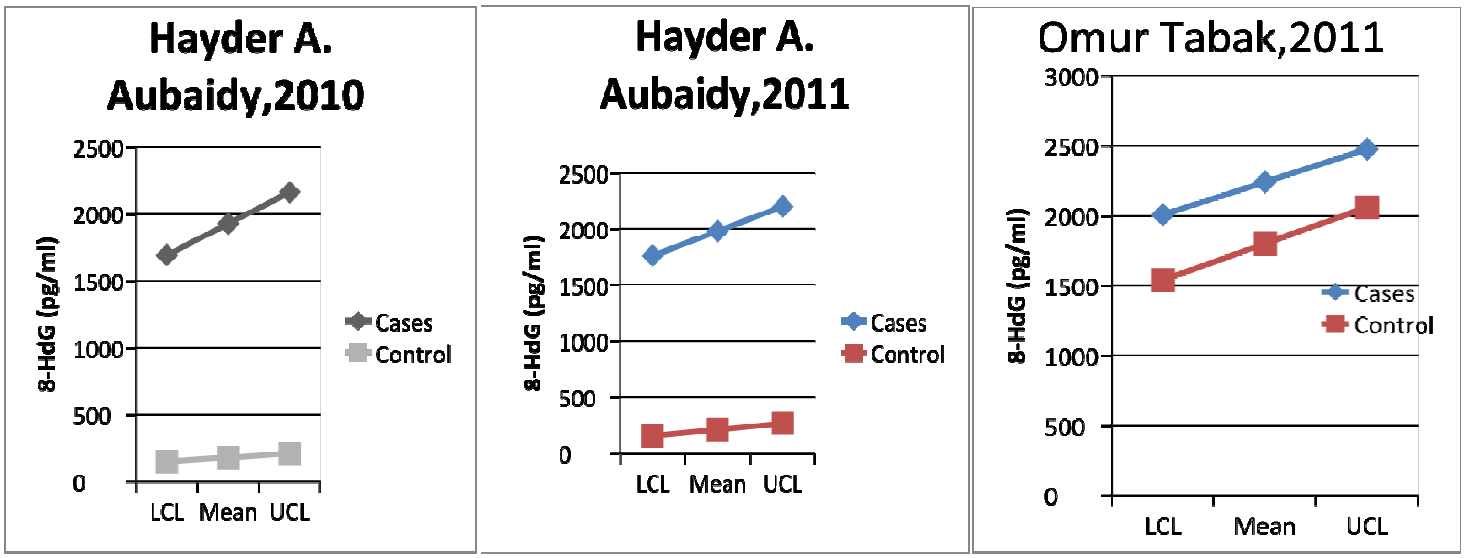
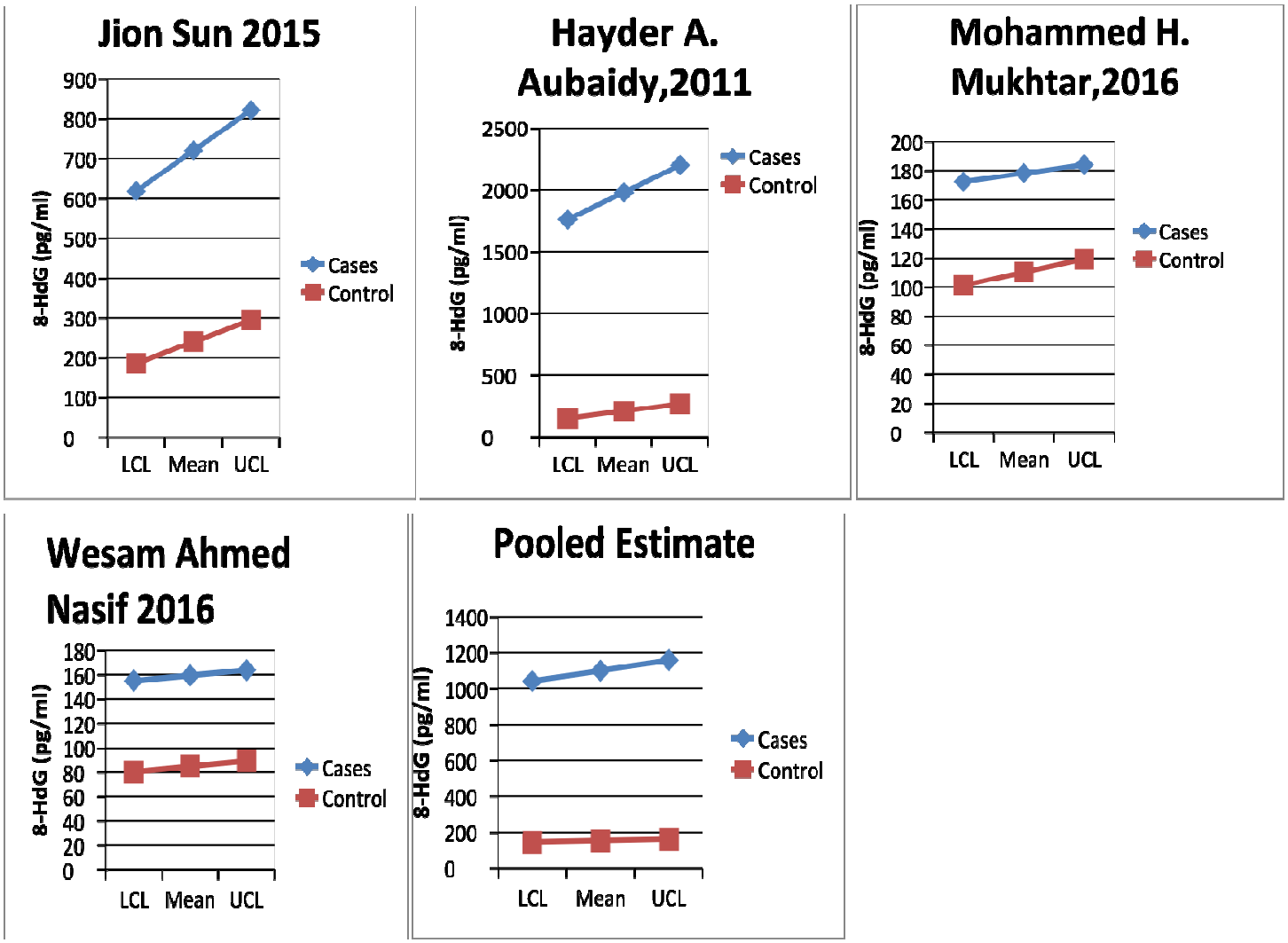
Table-2 Application of independent sample t test to individual studies with aggregate estimate by weighted average method.

Sr.No.	Author/ Year	"t" Cal.	Degree of freedom (df)	Level of significance
1	Hayder A. Aubaidy 2010[14]	8.76	131	p<0.0000
2	Hayder A. Aubaidy 2011 [15]	8.61	152	p<0.0000
3	Omur Tabak 2011 [16]	1.89	86	p<0.06
4	Jion Sun 2015 [17]	6.03	91	p<0.0000
5	Hyder A Aubaidy2011 [18]	8.54	79	p<0.0000
6	Mohammed H. Mukhtar 2016 [19]	13.34	128	p<0.00000
7	Wesam Ahmed Nasif 2016 [20]	23.58	98	p<0.00000
	Pooled Estimate	**24.3**	**777**	**P=0.0000000….**

Figure 2- Linear graphs of 95% confidence intervals of 8-HdG levels of cases of T2DM and comparable healthy controls



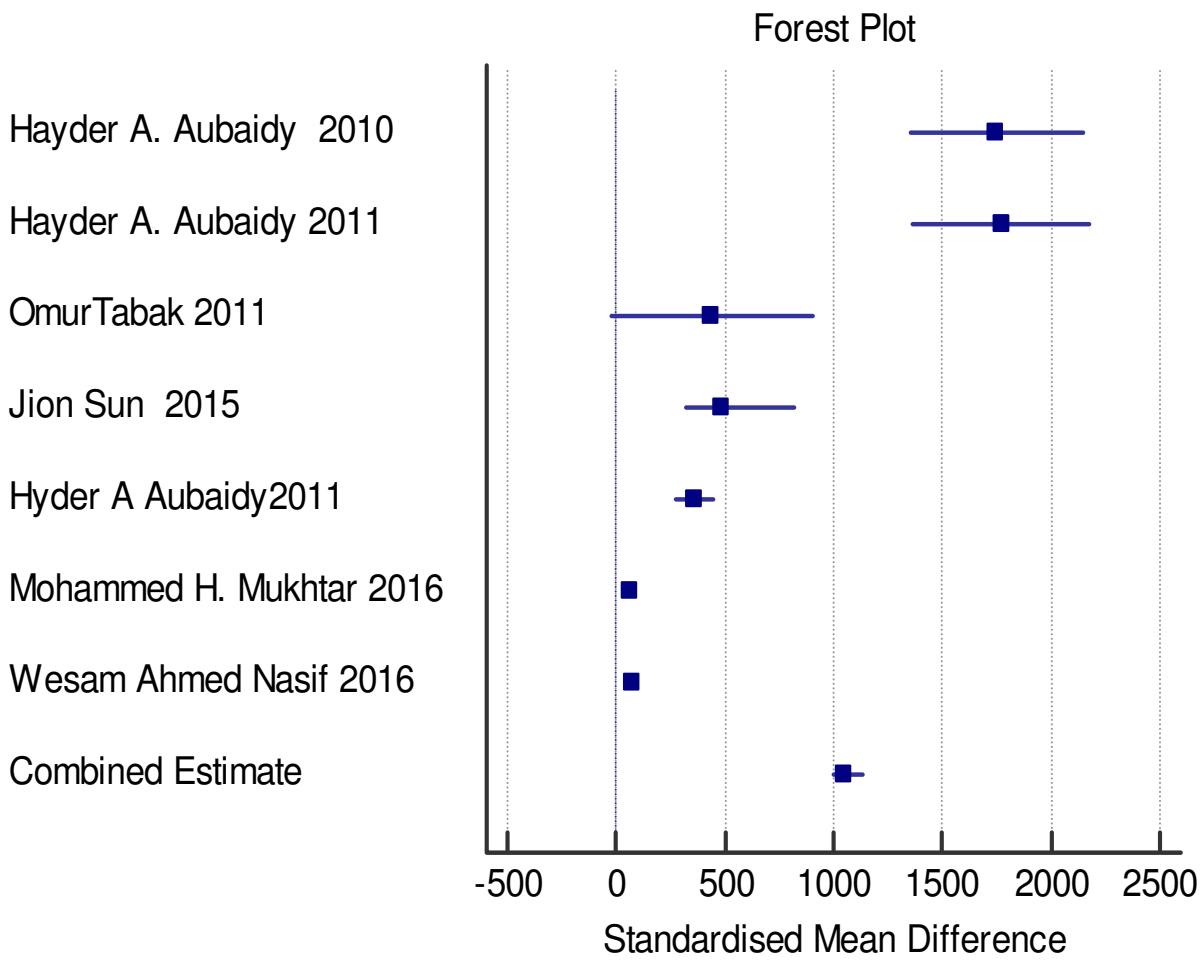


Forest plot for 8-HdG shows a significantly increased 8-HdG level in T2DM cases compared to controls,

The overall standard mean deference (SMD) and (95% CI) was1051.06 (+996.29 to +1135.83) respectively, (p=0.0000000)**.**

(Fig 3 & Table 3)

Figer-3 Forest plot showing the levels of 8-HdG in T2DM and healthy control individuals and combined study.



(SMD): +1051.06 [95% CI (+996.29 to +1135.83)] (p=0.0000000).

Table-3 Levels of 8HdG in T2DM cases and healthy control individuals and combined estimate.

Sr.No.	Study	SMD (95% CI)	Weight %
1	Hayder A. Aubaidy 2010[14]	+1748.5 (+1355.49 to +2142.11)	17.07
2	Hayder A. Aubaidy 2011 [15]	+1769.2 (+1364.58 to +2173.82)	19.77
3	Omur Tabak 2011 [16]	+440 (-22.6 to +902.6)	11.30
4	Jion Sun 2015 [17]	+480 (+321.58 to+ 821.74)	11.94
5	Hyder A Aubaidy 2011 [18]	+359.5 (+275.68 to+ 443.32)	10.40
6	Mohammed H. Mukhtar 2016 [19]	+ 68.15 (+58.03 to+78.22)	16.69
7	Wesam Ahmed Nasif 2016 [20]	+74.51 (+68.25 to+ 80.77)	12.83
8	**Combined Estimate**	**+1051.06 (+996.29 to +1135.83)**	**100.00**

Discussion:-

To our knowledge, studies where the meta-analysis is done to assess the association between 8-HdG and T2DM are not commonly seen. In our meta-analysis the result showed that 8-HdG in the T2DM group was higher than that in healthy controls. (t=24.03, df=777 p=0.0000000 ….) This also shows extremely significant positive relationship between 8-HdG and risk of T2DM.

In the given seven studies there is a highly significant difference in average values of 8-HdG in cases and controls so combined estimate is extremely significant. However in study ***Omur Tabak 2011 et al*** considering pooled variance the difference is not significant (tcal =1.89 and p=0.06. not significant SMD +440, 95% CI (-22.6 to +902.6), however considering individual variance the difference is significant. Cases [2240 (95 % CI 2005.38 to 2474.62) and controls (1800 95% CI 1539.91 to 2060.09) that significant.

Reactive oxygen species (ROS), which is formed by hyperglycaemia, can damage nucleic acids, lipids, and proteins with parallel changes in the biochemically make-up of blood constituents. Increased levels of biomarkers or risk factors associated with oxidative damage to lipids, proteins and DNA have been detected in serum of diabetic patients and their presence is correlated with the development of diabetes complications including atherosclerosis [10, 21].

This is first meta-analysis to our best knowledge showing that serum 8-HdG is higher in T2DM than controls. In recent years, study indicates a rise in 8-OHdG levels from control to prediabetes and diabetes stage. 8-OHdG is an expression of DNA damage in endothelial tissue and oxidative stress [7,4]. Animal study has indicated that serum and urinary 8-HdG as oxidative stress marker in T2DM, resulting suggested that 8-HdG in urine and serum may be good biomarker [22]. ***Kakimota et al*** first time demonstrated that oxidative mtDNA damage and subsequent mtDNA deletion may be accumulated in Kidney of diabetic rats [23]. ***He et al*** showed study on Human also indicated that 8-HdG in urine and serum may oxidative trees biomarker for T2DM [8].

Elevated blood glucose levels are clearly responsible for microvascular complications of diabetes and the pathogenesis of atherosclerotic macrovascular disease. Hyperglycaemia leads to the formation of reactive oxygen species. These superoxide anions cause DNA strand breakage with an increase in 8-OHdG Sand destruction of endothelial function resulting in atherosclerosis [14]. Clinical relevance of elevated 8-OHdG levels are supported by previous studies with increases in 8-OHdG noted in skeletal muscle, monocular cells and from patient with type 2 diabetes in accordance with disease progression and DNA damaging leukocytes of type 2 diabetic patients compared to control subjects [10,24,25].

Inclusion and exclusion criteria were strictly adhered to. 8-HdG between the T2DM and non-diabetes mellitus group were compared. Significant positive association between and 8-HdG T2DM was observed.

Limitations: Very few studies fulfilling all the eligibility criteria were identified. Only single variable like serum 8-HdG was involved in the analysis. Urine 8-HdG study excluded. Due to non-availability of the suitable data we could not estimate Odd's Ratio/ Risk Ratio.

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Conclusion:-

The current study shows that increased8-HdG is the predictive bio-marker of the DNA damage an oxidative stress biomarker for T2DM. Further 8-HdG 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 monitoring for T2DM.

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