RESEARCH ARTICLE

STUDY OF FACTORS LIKE SMOKING AND ITS ASSOCIATION WITH INFLAMMATION AND CARDIOVASCULAR RISKS IN PRE-DIABETIC AND DIABETIC PATIENTS.

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Abstract

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Background: Cigarette consumption increases both the risk of diabetes and diabetic vascular complications. It is associated with dyslipidemia, insulin resistance and inflammation; however the exact mechanisms are still to be elucidated. In this study we aimed to compare the levels of inflammatory markers and assess the cardiovascular risks in terms of CRR, AI, AC and AIP among the patients with pre-diabetes and diabetes.

Method: This study included 100 control subjects, 145 pre-diabetic and 126 diabetic patients. The patients were further categorised into non smoker (44 pre-diabetes and 40 diabetes) and smoker (101 pre-diabetes and 86diabetes) groups. Inflammatory biomarkers likefibrinogen, CRP, IL-6, adiponectin and uric acid were assayed with standard kit methods.

Result: We observed high levels of fibrinogen, CRP, IL-6 and uric acid, and low level of adiponectin in pre-diabetic and diabetic smoker group compared to their non smoker counterparts but the level of statistical significance was achieved in case of adiponectin only. Pre-diabetic smokers also demonstrated significantly high fibrinogen compared to pre-diabetic non smokers. We also found high cardiovascular risk in pre-diabetic and diabetic individuals irrespective of smoking status. Further we documented significant association of inflammatory markers with cardiac risk indices especially in case of diabetic patients and the association was more profound in the patients who smoked.

Conclusion: Thus from this study it is evident that both pre-diabetic and diabetic individuals are highly susceptible to CVD risks induced by smoking. Diabetic individuals are further at a great threat compared to pre-diabetic individuals. So quiting of smoking can reverse the unwanted health outcomes of diabetes.

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Introduction:-

Smoking not only is the well known risk factor of diabetes but also is the important agent to deteriorate diabetic status leading to end organ damage via increased production of free radicals and inflammatory molecules. A number of studies have documented smoking induced altered metabolism of glucose and lipid in diabetic patients. Smoking increases insulin resistance that culminates hypergycemia and dyslipidemia. These studies also implicated the

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requirement of large dose of insulin in diabetic smokers compared o non smokers to avail similar metabolic outcome [1]. In an experimental study (glucose-clamp method), infusion of nicotine in both healthy and diabetic volunteers showed that in healthy volunteers there was no difference in concentration of serum insulin while in case of diabetic patients higher dose of insulin was required [2,3].Nicotine has negative effect on insulin release and its action, suggesting nicotine to be one of the prominent causes of insulin resistance. Further nicotine induces loss of pancreatic β-cells via oxidative stress, mitochondrial dysfunction and inflammation [4].

Chemicals present in cigarette smoke promote cellular injury thereby interferring its function and leading to inflammation. From the previous researches, it is evident that both oxidative stress and inflammation increase the diabetic risk. Diabetic patients who smoke are more prone to adverse health effects viz heart and renal diseases, diabetic foot infections or ulcers, retinopathy and peripheral neuropathy compared to non smoker diabetic patients. Moreover smoking has been shown to have significant contribution towards the mortality of diabetic patients due to CVD and CHD (Coronary Heart Disease). On an average smokers are found to die 8-10 years earlier than those patients who do not smoke. It has been shown that the risk of CVD increases by 61% in those smokers who consume more than 20 cigarettes per day, 29% in smokers taking less than 20 cigarettes per day and 23% in the former smokers [5].

Thus the main objective of this study was to determine the association of smoking with inflammatory markers and the risk factors of cardiovascular diseases in pre-diabetic and diabetic patients so that our result may serve as mediator to promote cessation of smoking in diabetic patients in order to prevent blooming of diabetic vascular complications.

Materials And Methods:-

This case control study commenced in JLN MEDICAL COLLEGE & ATTACHED GROUP OF HOSPITALS with 100 healthy controls, 145 pre-diabetic patients and 126 diabetic patients. We enrolled the patients withhistory of inflammatory diseases, cardiovascular diseases pregnancy etc that affect the concentration of inflammatory markers. Before commencement approval from the ethical board of institution was obtained.

Each participant keenly participated and gave the written consents in this research. Age, BMI (Body mass index), WHR (Waist hip ratio) of each participants were recorded. Biochemical parameters such as fasting glucose, glycosylated haemoglobin (HbA1c), cholesterol, HDL, triglyceride (TG) and inflammatory parameters like adiponectin, fibrinogen, CRP, IL-6 and uric acid were estimated in each patient.

Fasting glucose was measured by GODPOD method, HbA1c was analysed using ion exchange resin method. Likewise total cholesterol, HDL and TG were analysed by CHOD-POD, CHOD-POD/phosphotungustate method and GPO-PAP method respectively. LDL was computed from Friedwald's equation [6] i.e. LDL=Total Cholesterol-[HDL+VLDL] where VLDL=TG/5. Inflammatory biomarkers like adiponectin and IL-6 were assessed by ELISA while CRP and fibrinogen were estimated respectively by immune-turbidimetric method. Uric acid was detected by uricase method.

The cardiac risk indices [7] were calculated as:
Calculation of cardiac risk indices/atherogenic indices was done as [12]:
1. Casteli's risk index (CRR I or CRI)

$$\text{Casteli's ris k index I} = \frac{Total\ cholesterol}{HDL}$$

2. Casteli's risk index II (CRR II or CRII) or Atherogenic index (AI)

$$\text{Casteli's ris k index II} = \frac{LDL}{HDL}$$

3. Atherogenic coefficient (AC)

$$Atherogenic\ coefficient = \frac{Total\ cholesterol}{HDL}$$

4. Atherogenic index of plasma (AIP)

$$Atherogenic\ index\ of\ plasma = \frac{Cholesterol - HDL}{HDL}$$

Statistical analysis
The concentration of study parameters (age, WHR, BMI, fasting sugar, HbA1c, cholesterol, TG, HDL, LDL, adiponectin, fibrinogen, CRP, IL-6 and uric acid) were expressed as mean±sd. The difference in the level of these parameters among control and pre-diabetic groups, control and diabetic groups, and pre-diabetic and diabetic groups were investigated by student's t test. The association between inflammatory mediators and other parameters included was determined by Pearson's correlation coefficient. The p<0.05 was implicated to be statistically significant.

Results:-
In table 1 the comparison of basic parameters among the three groups namely control, pre-diabetic and diabetic groups is shown. The pre-diabetic and diabetic groups were further categorised base on their smoking habit. In table 2 comparisons of basic parameters between non smoker and smoker is shown. There was no significant difference in the level of basic parameters when compared between pre-diabetic non smoker/smoker groups and diabetic non smoker/smoker groups. However when the biochemical parameters were compared between pre-diabetic versus diabetic non smoker groups and pre-diabetic versus diabetic smoker groups, significantly high values of glucose, HbA1c and lipid parameters (LDL, TG) were obtained but the difference in HDL was significant only in case of pre-diabetic versus diabetic non smoker group. Non smoker diabetic subjects had low HDL level compared to non smoker pre-diabetic subjects.

In this study we evaluated levels of inflammatory markers in both non smoker and smoker categories of pre-diabetic and diabetic patients. Table 3 represents comparison between control, pre-diabetic and diabetic groups. Table 4 represents the comparative analysis among pre-diabetic and diabetic non smoker and smoker group. Significantly high adiponectin and significantly low fibrinogen levels in pre-diabetic non smoker patients compared to pre-diabetic smoker patients. Similarly significantly low adiponectin was observed in diabetic patients who smoked compared to the diabetic non smokers. When comparison was made between pre-diabetic/diabetic non smokers and pre-diabetic/diabetic smokers, significantly high values of fibrinogen, CRP, IL-6 and uric acid, and significantly low level of adiponectin were observed in case of patients of diabetic category (non smoker and smoker).

In tables 5 and 6, cardiac risk indices were assessed among the three study groups (control, pre-diabetic and diabetic) as well as the non smoker and smoker category of pre-diabetic and diabetic patients. The risk of CVD was significantly high in both pre-diabetic and diabetic subjects as indicated by higher values of cardiac risk indices compared to control group (table 5). However when comparison was made among pre-diabetic non smoker/smoker patients and diabetic non smoker and smoker patients, the level of cardiac risk indices were high in pre-diabetic and diabetic patients who smoked. In case of pre-diabetic versus diabetic non smoker and smoker categories diabetic patients showed significant increase in the level of all the indices (table 6).

The inflammatory markers were correlated with the cardiac risk indices and the correlation was statistically significant irrespective of smoking habit of pre-diabetic and diabetic individuals (table 7 and 8).

Table 1:-Comparison basic parameters in Control, Pre-diabetic groups and Diabetes

Parameter	Control C	Pre-diabetes (P)	Diabetes (D)	p(C/P)	p(C/D)	p(P/D)
Age	42.87±7.87	48.04±6.78	49.67±10.26	<0.001**	<0.001**	0.09
BMI	23.42±2.1	23.99±2.4	24.35±2.72	0.03*	<0.003**	0.27
WHR	0.85±0.08	0.91±0.12	0.9±0.12	<0.001**	<0.001**	0.67
Glucose	84.53±7.24	116.63±5.15	160.49±40.15	<0.001**	<0.001**	<0.001**
HbA1c	5.05±0.53	5.87±0.44	6.36±0.89	<0.001**	<0.001**	<0.001**
CHO	173.27±13.58	186.64±25.98	195.59±35.68	<0.001**	<0.001**	0.01**
HDL	49±5.18	48.19±4.49	46.12±4.73	0.19	<0.001**	0.004**
TG	105±21.27	110.37±20.96	123.1±37.44	0.04*	<0.001**	<0.001**
LDL	103.1±13.2	116.28±28.21	124.8±35.14	<0.001**	<0.001**	0.008**

Statistically significant: *→p>0.05   **→p<0.01

Table 2:-Comparison basic parameters Pre-diabetic and Diabetic non smoker and smoker groups

Parameter	Pre-diabetes	Pre-diabetes	Diabetes	Diabetes (Smoker)

	(Non smoker)	(Smoker)	(Non smoker)	
Age	48.3±6.49[a]	47.41±7.44[c]	49.36±9.93[b]	50.35±11.03[d]
BMI	24.03±2.53[a]	23.87±2.06[c]	24.3±2.74[b]	24.4±2.75[d]
WHR	0.91±0.12[a]	0.89±0.1[c]	0.91±0.13[b]	0.87±0.11[d]
Glucose	117.28±4.92[a*]	115.14±5.43[c**]	160.11±43.53[b**]	161.31±32.19[d]
HbA1c	5.85±0.47[a]	5.9±0.37[c**]	6.34±0.88[b**]	6.41±0.92[d]
CHO	186.86±26.96[a]	186.1±23.87[c]	196.2±36.61[b]	194.2±34.02[d]
HDL	48.3±4.26[a]	48±5.02[c]	45.87±4.61[b**]	46.65±5[d]
TG	112.9±21.99[a]	105.93±17.8[c**]	123.67±40.94[b*]	121.88±28.92[d]
LDL	116±28.99[a]	116.9±26.65[c]	125.61±35.15[b*]	123.19±35.51[d]

Statistically significant: *→p>0.05   **→p<0.01

a→Pre-diabetic non smoker vs smoker

b→Pre-diabetic non smoker vs diabetic non smoker

c→Pre-diabetic smoker vs diabetic smoker

d→Diabetic non smoker vs diabetic smoker

Table 3:-Comparison of markers of inflammation in Control, Pre-diabetic and Diabetic groups

Parameters	Control C	Pre-diabetes (P)	Diabetes (D)	p(C/P)	p(C/D)	p(P/D)
Adiponectin	9.01±2.82	8.15±1.87	6.84±1.98	<0.001**	<0.001**	0.04*
CRP	2.81±1.13	4.17±1.36	5.15±1.73	<0.001**	<0.001**	<0.001**
IL-6	4.31±1.8	5.87±1.6	7.51±2.25	<0.001**	<0.001**	<0.001**
Fibrinogen	331.18±58.61	346.58±55.78	369.6±61.38	0.03*	<0.001**	0.001**
Uric acid	4.47±0.76	4.64±1.04	6.33±1.89	0.12	<0.001**	<0.001**

Statistically significant: *→p>0.05   **→p<0.01

Table 4:-Comparison of markers of inflammation in Pre-diabetic and Diabetic groups non smoker and smoker groups

Parameter	Pre-diabetes (Non smoker)	Pre-diabetes (Smoker)	Diabetes (Non smoker)	Diabetes (Smoker)
Adiponectin	8.36±1.95[a*]	7.67±1.58[c**]	7.03±2.27[b**]	6.43±1.02[d*]
CRP	4.16±1.43[a]	4.18±1.21[c**]	5.1±1.77[b**]	5.26±1.67[d]
IL-6	5.84±1.61[a]	5.94±1.57[c**]	7.4±2.33[b**]	7.75±2.07[d]
Fibrinogen	340.45±55.79[a*]	360.68±43.89[c]	367.36±59.43[b**]	374.42±65.9[d]
Uric acid	4.69±1.04[a]	4.53±1.04[c**]	6.27±1.88[b**]	6.46±1.93[d]

Statistically significant: *→p>0.05   **→p<0.01

a→Pre-diabetic non smoker vs smoker

b→Pre-diabetic non smoker vs diabetic non smoker

c→Pre-diabetic smoker vs diabetic smoker

d→Diabetic non smoker vs diabetic smoker

Table 5:-Comparison of cardiac risk indices in control, pre-diabetic and diabetic group

Parameter	Control C	Pre-diabetes (P)	Diabetes (D)	p(C/P)	p(C/D)	p(P/D)
CRR	3.6±0.5	3.92±0.82	4.32±1.09	<0.001**	<0.001**	0.002**
AI	2.1±0.4	2.46±0.78	2.77±0.97	<0.001**	<0.001**	0.004**
AC	2.58±0.53	2.93±0.82	3.32±1.09	<0.001**	<0.001**	0.001**
AIP	0.32±0.1	0.35±0.09	0.41±0.15	<0.02*	<0.001**	<0.001**

Statistically significant: *→p>0.05   **→p<0.01

Table 6:-Comparison of cardiac risk indices in pre-diabetic and diabetic non smoker and smoker groups

Parameter	Pre-diabetes (Non smoker)	Pre-diabetes (Smoker)	Diabetes (Non smoker)	Diabetes (Smoker)
CRR	3.91±0.82[a]	3.94±0.83[c**]	4.23±0.94[b**]	4.365±1.16[d]
AI	2.451±0.78[a]	2.49±0.78[c*]	2.696±0.88[b**]	2.814±1.02[d]

| AC | 2.93±0.82[a] | 2.94±0.83[c**] | 3.23±0.94[b**] | 3.37±1.16[d] |
| AIP | 0.36±0.1[a] | 0.339±0.09[c*] | 0.41±0.13[b**] | 0.41±0.15[d] |

Statistically significant: *→p>0.05   **→p<0.01
a→Pre-diabetic non smoker vs smoker
b→Pre-diabetic non smoker vs diabetic non smoker
c→Pre-diabetic smoker vs diabetic smoker
d→Diabetic non smoker vs diabetic smoker

Table 7:-Correlationof inflammatory markers with cardiac risk factors in pre-diabetic non smoker and smoker groups

Parameter	Pre-diabetic Non smoker				Pre-diabetic Smoker			
	CRR	AI	AC	AIP	CRR	AI	AC	AIP
Adiponectin	-0.25*	-0.23*	-0.27**	-0.34**	-0.44**	-0.44**	-0.44**	-0.25
CRP	0.21*	0.21*	0.22*	0.21*	0.27	0.25	0.27	0.2
IL-6	0.15	0.12	0.16	0.35**	0.01	0.03	0.01	0.23
Fibrinogen	0.17	0.18	0.21*	0.15	0.5**	0.48**	0.5**	0.44**
Uric acid	0.18	0.15	0.16	0.11	0.07	0.07	0.07	0.06

Statistically significant: *→p>0.05   **→p<0.01

Table 8:-Correlationof inflammatory markers with cardiac risk factors in diabetic non smoker and smoker groups

Parameter	Diabetic Non smoker				Diabetic Smoker			
	CRR	AI	AC	AIP	CRR	AI	AC	AIP
Adiponectin	-0.3**	-0.22	-0.3**	-0.51**	-0.5**	-0.47**	-0.5**	-0.54**
CRP	0.73**	0.69**	0.73**	0.62**	0.66**	0.62**	0.66**	0.55**
IL-6	0.44**	0.42**	0.44**	0.43**	0.54**	0.48**	0.54**	0.61**
Fibrinogen	0.31**	0.32**	0.31**	0.15	0.4*	0.36*	0.4*	0.37*
Uric acid	0.26*	0.23*	0.01	0.31**	0.39	0.42**	0.39*	0.26*

Statistically significant: *→p>0.05   **→p<0.01

Discussion:-
Smoking increases diabetes incidence in a dose dependent manner. Several cohort studies have demonstrated increased risk of diabetes induced by smoking. A four year follow up study of Cho *etal* reported significantly elevated diabetic risk in both past and current smokers. The authors also suggested an increase in the risk with the increase in number of cigarette intake [8]. Similarly, in another 14 years long cohort study, it was shown that risk of diabetes was 1.55 among the individuals who smoked ≥20 cigarettes/day compared to the non smokers [9]. Further, in another follow up study [10] and a British study [11], the risk of diabetes was reported to 1.94 and 1.7 respectively.

The exact mechanism on how smoking increases diabetic risk though unclear, it is depicted that smoking induces increase in hyperglycemic hormones like epinephrine and nor epinephrine that may contribute to blood glucose by promoting hepatic glycogenolysis and gluconeogenesis [12]. Further smoking causes generation of free radicals that promote lipid peroxidation, DNA damage and protein oxidation thereby leading to pancreatic β-cell apoptosis [13].Oxidative stress induced due to smoking is also responsible for the systemic inflammation and endothelial dysfunction observed in diabetic patients. Also smoking has been implicated to contribute to dyslipidemia which is the major attributor to atherosclerosis. Smokers are shown to have high TG, FFA (free fatty acids), LDL and low HDL levels thereby suggesting pathological relationship between diabetic vascular disease and smoking [14].

In this study we evaluated levels of inflammatory markers like adiponectin, fibrinogen, CRP, IL-6 and uric acid in pre-diabetic and diabetic patients and compared that with the control groups. We also assessed cardiovascular risk in the form of cardiac risk indices and correlated them with inflammatory parameters in both pre-diabetic and diabetic non smoker and smoker groups. We did not find significant differences in the level of basic parameters when compared between pre-diabetic non smoker versus smoker groups and diabetic non smoker versus smoker groups. However on comparative analysis between pre-diabetic versus diabetic groups in both non smoker and smoker categories significant differences were observed in case of fasting glucose, HbA1c, HDL, TG and LDL. On analysing the levels of inflammatory markers among non smoker and smoker pre-diabetic and diabetic patients we

observed significantly low adiponectin level in both pre-diabetic and diabetic smoker groups compared to their non smoker counterparts. We could also document significantly high fibrinogen in pre-diabetic smokers compared to pre-diabetic non smokers. The level of IL-6, CRP and uric acid though were high in smoker group, the level of statistical significance could not be achieved in both the patients (pre-diabetic and diabetic).

Thamer C*etal* [15] and Kotani K[16] in their study showed decrease in adiponectin level due to smoking. Likewise,Levitzky YS*etal* [17] and Hastie CE *etal* [18] demonstrated positive association between CRP and smoking. According to Efstathiou SP*etal* smoking induces decrease in adiponectin is temporary since cessation of smoking facilitates increase in the level of serum adiponectin to the normal level [19]. One of the plausible mechanisms for smoking induced decrease in adiponectin level could be the oxidative stress that leads to the deactivation of PI-3 kinase (Phosphatidyl inositol kinase) which is a key protein for expression and secretion of adiponectin from adipocytes [20]. Further chronic smoking also causes increased production of TNF-α that promotes hypoadiponectinemia [21]. Nicotine present in smoke further reduces the expression of adiponectin mRNA in adipocytes [20].

Smoking is a chief contributor of CVD independent of diabetic status of the subject. The risk is further amplified if the subject is hyperglycaemic. A prospective 8 years follow up study showed significant association of CHD (Coronary heart disease) with smoking in diabetic patients. Similar results were obtained in the UKPD study too [22, 23]. The Nurses Health study demonstrated the dose dependent increase in CHD risk among diabetic women who smoked. The relative risks were depicted to be 1.66 and 2.68 for those who consumed 1-14 cigarettes/day and ≥15 cigarettes/day respectively [24]. Similarly, a meta-analysis study demonstrated hazard ration of 1.42 for CHD in diabetic patients who were current smokers compared to the non smokers. The authors also suggested that cessation of smoking can culminate greater benefits to diabetic patients by lowering the burden of CVD [25]. Keeping this in view we assessed the future risk of CVD in patients with pre-diabetes and diabetes by evaluating various cardiac risk indices such as CRR, AI, AC and AIP with respect to smoking status. Independent of smoking status we found significantly high cardiac risk indices in patients with pre-diabetes and diabetes compared to controls. On categorising the patients based on smoking status, increased levels of cardiac risk indices were observed in pre-diabetic smokers and diabetic smokers compared to their non smoker counterparts, however the increase was not significant statistically. Significantly high values were documented in diabetic non smoker and smoker groups when comparison was made with the respective non smoker counterparts.

Since smoking also induces systemic inflammation that contributes to diabetes and CVD, in this study we determined the association between inflammatory mediators and cardiac risk indices. In case of pre-diabetic smoker group, adiponectin and fibrinogen showed significant negative and positive association while in case of pre-diabetic non smoker group such association was observed in case of adiponectin and CRP. In case of diabetic patients, all the assayed inflammatory parameters were significantly correlated with cardiac risk indices and the correlation was more pronounced in diabetic patients who smoked. The correlation with adiponectin was negative while with fibrinogen, CRP, IL-6 and uric acid was positive.

Conclusion:-
Smoking exerts harmful effect to diabetic patients by increasing CV mortality. Smoking promotes insulin resistance, inversely affects insulin function, cause oxidative damage and induce inflammatory phenomena all of which are key to diabetic complications especially macro vascular ones. The result of our study also supported this fact as we observed elevated cardiovascular risks and significant association of inflammatory mediators with these risk factors (cardiac risk indices) in pre-diabetic and diabetic subjects. Thus, it is recommended that these patients should be educated on the adverse outcomes of smoking and encouraged to quit smoking, since cessation of smoking can also restore the level of cardio-protective cytokine adiponectin. Also further studies are to be sought regarding effect of cigarette smoke on glucose metabolism and inflammatory responses leading to development of CVD as there is paucity of researches in this regard so that burden to diabetic epidemiology can be alleviated.

Conflict of Interest:
None declared.

Research funding:
None declared.

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