RESEARCH ARTICLE

A CORRELATIVE STUDY OF SERUM ADENOSINE DEAMINASE, MALONDIALDEHYDE, ADVANCED OXIDATION PROTEIN PRODUCTS AND SPIROMETRIC ANALYSIS IN SMOKERS

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Manuscript Info	*Abstract*
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	Introduction: Smoking is most common among the men aged 15 and above accounting for 51% of the population. The rate at which the adolescent boys use tobacco is 18% globally [GHO, WHO]. In India, an estimated 82.3% males with COPD are smokers. Oxidative effect via free radical generation in smokers has been widely investigated, as it causes the lipid peroxidation, damage to tissues primarily Lung apart from the cardiac diseases. **Objective(s) of Study:** To estimate the serum levels of Adenosine Deaminase (ADA), Malondialdehyde (MDA), advanced oxidation protein products (AOPP) in smokers, & correlate with Spirometric changes in them. **Material and Method:** This is a crossectional observational analytical study conducted for a duration of six months at a Medical College Hospital in Mangalore city. A total number of eighty healthy individuals who visited medicine opd were included in this study. Out of these subjects, forty were smokers and other forty nonsmokers. **Results:** The results were analyzed in the SPSS-23version. A significnat high level of serum MDA, AOPP and low levels of ADA was observed in smokers when compred to non-smokers. Spirometric analysis showed statistical significant decrease in FEV_1 ($58.5\pm12\%$) in smokers. A significant inverse relation of the MDA, AOPP with Spirometric changes & positive relation of ADA levels with spirometry was also observed. **Discussion & Conclusion:** From this study, it is clear that the smokers are at a high risk of pulmonary diseases. The utility of these parameters as markers for the oxidative damage and variation in lung function test may be a useful tool in educating smokers regarding the ill effects with possible health derangement & urge them to quit smoking.

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Introduction:-
Smoking is most common among the men aged 15 and above accounting for 51% of the population. Globally about 18% of adolescent boys use tobacco [GHO, WHO]. Smoking is one of the most important risk factor for developing the chronic obstructive pulmonary disease (COPD).[1] In India, an estimated 82.3% males with COPD are smokers.[2]

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It is a very old practice of the people all over the globe cutting across all the national and social barriers, and is one of the widely accepted social habits. Increase in the habit of cigarette smoking has led to high-incidence of the diseases related to it in developed as well as the developing nations.

Oxidative stress via free radical generation in smokers has been widely investigated, as they cause lipid peroxidation, oxidation of proteins and even damage to tissues mainly Lung.[3] Cigarette smoking induces lung inflammation, repeated injury and followed by repair. The oxidative stress and an excess of proteinase in lung further modify lung inflammation. This increase in oxidative stress can be seen as the change in levels of lipid peroxidation and oxidative protein products. Malondialdehyde (MDA) is the most important marker for assessing the free radical-induced lipid peroxidation[4] and Advanced Oxidation Protein Product (AOPP)[5] and Adenosine Deaminase (ADA) are useful in assessing the oxidative damage. Smokers are at risk of alveolar damage leading to pulmonary disorders even without the pulmonary symptoms a person may have inflammatory damage to the alveolar cells and at times leading to reduced lung capacity. It is proven that smoking is one among the major and strong risk factor for the cardiac and pulmonary diseases.[6] Hence it is a greater responsibility of the treating physicians to combat with the smoking habits, both through their strict advice to the patients and through the influence of the society/ community by health education for quitting the habit of cigarette smoking and creating awareness of its benefits.

Objectives of Study:-
To estimate the serum levels of Adenosine Deaminase (ADA), Malondialdehyde (MDA), Advanced Oxidation Protein Products (AOPP) in smokers and to correlate these parameters with Spirometric changes.

Materials and Method:-
This is a crossectional observational analytical study, conducted at a Medical College Hospital in Mangalore city, Karnataka. A total of eighty apparently healthy subjects who visited medicine OPD for health checkup were included in this study. Out of these subjects, forty were smokers and other forty were non-smokers. The study was started after taking the clearance from the ethics committee of the institute. The required sample size was calculated by SPSS sample size calculator keeping the power of study >80% and significance p-value <.05.

Blood Specimen:
About 5 ml venous blood was collected from the subjects with proper aseptic precautions after obtaining informed consent from the included subjects. Within one hour of samples drawn, it was subjected for centrifugation at the rpm of 3000 for about 10-15mins. The serum was then separated and stored in aliquots at -20℃ for a period less than 3 months before analyzing.

The following parameters were analyzed in serum by spectrophotometric methods, in UV visible spectrophotometer (Systronics 117).

Serum ADA was measured by photometric, Endpoint by the commercial kit by tulip diagnostics ADA-MTB, the procedure is followed as standardized in kit literature.[7]

Serum MDA was quantified by Thiobarbituric acid (TBA) method[10] based on ohkawa et al. 0.75ml serum was added to 3ml of MDA reagent (75mg Thiobarbituric acid +15gm trichloroacetic acid, in 2.08mL of 0.2N HCl, made up to 100mL with distilled water) added, mixed and kept in boiling water bath for 20 minutes. Then cooled under tap water, centrifuged at 3000 rpm for 10 minutes, and absorbance was measured at 535 nm against reagent blank. The level of MDA was calculated using the molar extinction coefficient of the MDA-Thiobarbituric acid complex.

Serum AOPP was measured by modified AOPP method (mAOPP)[9] 320 microlitre of the sample was treated with 8 microlitre of MgCl2 (Magnesium Chloride) and 32 microlitre of PTA (Phosphotungstic acid), then centrifuged at 5000 rpm for 20 minutes. Then 200 micro litre of the supernatant was taken and then 800 microlitre of Phosphate Buffered Saline, 50 microlitre of 1.16 M potassium iodide and 100 microlitre of acetic acid are added to each tube and absorbance at 340 nm was measured immediately. The concentration of AOPP expressed in chloramine T equivalent.

Spirometry was done in smokers and controls, after taking required precautions and adequate patient preparation as needed. Spirometric analysis conducted by using spirometer EasyOne[TM].[(10)] : the testing was done by the standard maneuver, with three distinct phases to the FVC:
1. Maximal inspiration
2. A "blast" of exhalation
3. Continued complete exhalation to the end of the test (EOT)

All the steps were followed according to the method mentioned in the manual and results were recorded. The FEV1 recorded by the test method was taken as an indicator of the total lung capacity status in the study subjects. Other indicators were FVC, FEV_1/FVC ratio.

Inclusion criteria:
Males aged 18-60yr with cigarette smoking history of more than a year and more than 100 cigarettes/bidi till date. Healthy controls who are non-smokers (with history of less than 100 cigarettes/bidi smoked in lifetime till date), and aged between 18-60yr.

Exclusion criteria:
Individuals with past or family history of Asthma, Pulmonary tuberculosis, Diabetes Mellitus, Chronic Renal Failure, Liver diseases, Tobacco chewer, Occasional smokers, Carcinomas. Female and trans-gender (as it is difficult to get the statistical number in the region).

Statistical analysis:
The data is represented as the Mean, SD, range, mean difference between the groups was measured using student 2-tailed independent t-test, the strength of correlation among the change in variable levels b using Pearson's correlation and mean difference between the groups of cigarette pack-year group using the ANOVA and post hoc analysis. The statistics was performed using the sophisticated IBM SPSS software package version23.

Results:-
The study was divided into two groups based on the smoking habit. Total of 80 healthy males were recruited for the study and divided into; Case – Cigarette Smokers, Control – Non-Smokers.

The mean age difference between the smokers and non-smokers was found to be nonsignificant and the result summary of the various parameters measured in the smokers are represented in table 2 and mean difference between the groups. Table 3 showing the strength of relation between the variables in the smokers, table 4 showing the mean pack of cigarette smoking per day by the various study subjects and the variation in levels of the parameters and significant difference in the mean.

Table 1:- Demonstrating the age of subjects in smokers and non-smokers as mean & SD.

		Smoking	
		Non-smokers (n=40)	Smokers (n=40)
Age, (years)	Mean	45.4	46.1
	SD	12.0	11.0

Table 2:- Demonstrating the mean and SD of the analyzed parameters in smokers and non-smokers& t-test result:

Result summary	Non-Smoker (n=40)	Smoker (n=40)	sig
ADA (U/L)	19.28 ± 5.48	15.40 ± 5.67	.001 HS
MDA (nmol/dL)	301.23 ± 100.39	926.61 ± 253.57	.001 HS
AOPP(ChloramineT equivalent)	46.26 ± 30.07	88.94 ± 44.54	.001 HS
SPIROMETRY-FEV_1 (%)	77.6 ± 7.9	58.5 ± 12.0	.001 HS

The Significance p-value <.05, HS- highly significant <.001

Table 3:- Demonstrating the Pearson correlation within the smoker group. The significant level is less than 0.05 p value.

Correlations-pearsons	Cigarette	Spirometry	ADA	MDA

Spirometry	r	-.666[**]			
	p-value	.000			
Sr ADA	r	-.296[**]	.260[*]		
	p-value	.007	.018		
Sr MDA	r	.922[**]	-.604[**]	-.289[**]	
	p-value	.000	.000	.009	
Sr AOPP	r	.525[**]	-.434[**]	-.039	.406[**]
	p-value	.000	.000	.731	.000

**. Correlation is significant at the 0.01 level (2-tailed).

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

Table 4:- Showing the mean ± SD of the study subjects in different groups based on number of packs cigarette per day and ANOVA with post hoc test for the mean difference.

	Non smoker (n=40) (a)	1 to 2 pack per day (n=7) (b)	2 to 3 pack per day (n=22) (c)	3 to 4 pack per day (n=11) (d)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
MDA	301.23±100.3	598.88±95.27 [a*]	918.33±217.32 [a,b*]	1151.74±122.69 [a,b,c*]
mAOPP	46.26±30.1	72.42±34.97	86.28±54.26 [a*]	104.76±18.42[a*]
ADA	19.28±5.48 [c*]	16.29±5.69	14.64±5.80	16.35±5.70
SPIROMETRY	77.6±7.9 [b,c,d*]	61.6±7.5	58.3±12.2	56.7±14.1

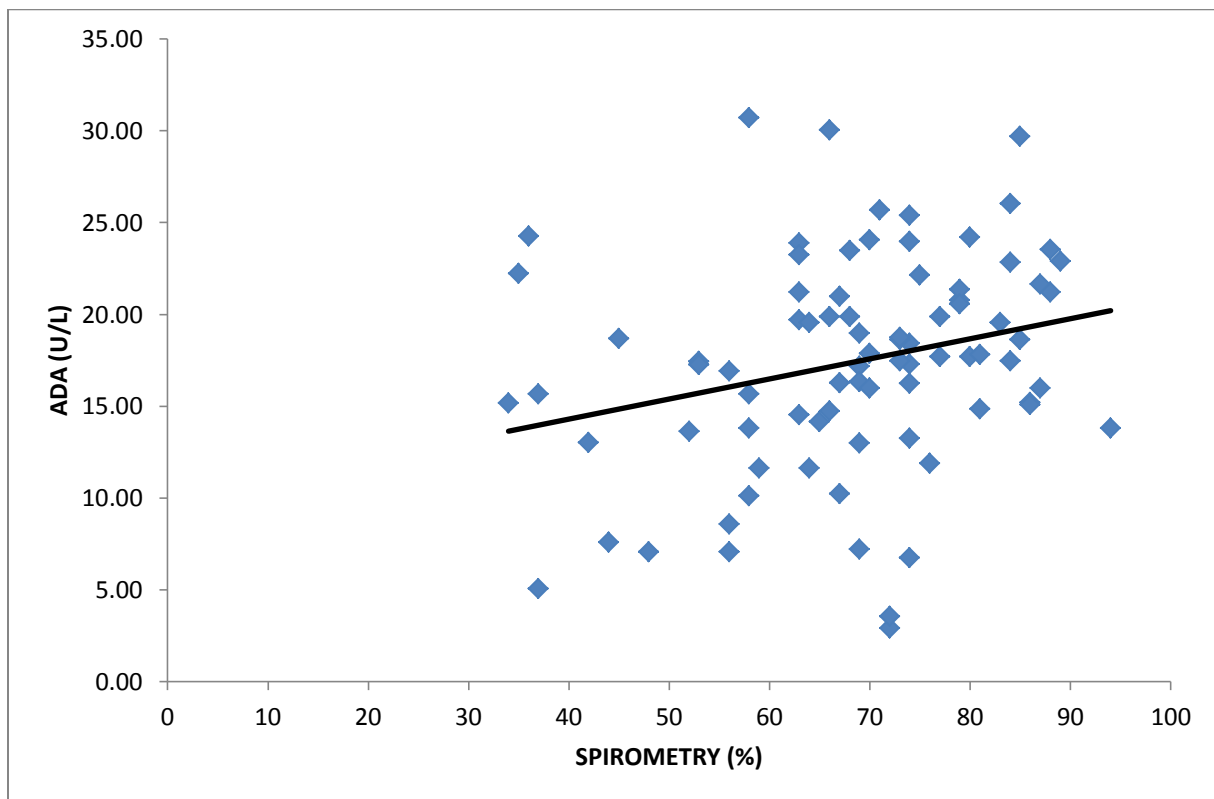*p-value <0.05 was considered as statistically significant.

Figure 1:- Scatter plot showing the relation between Adenosine Deaminase and Spirometry changes in study subjects (r= +0.260, p-value 0.018).
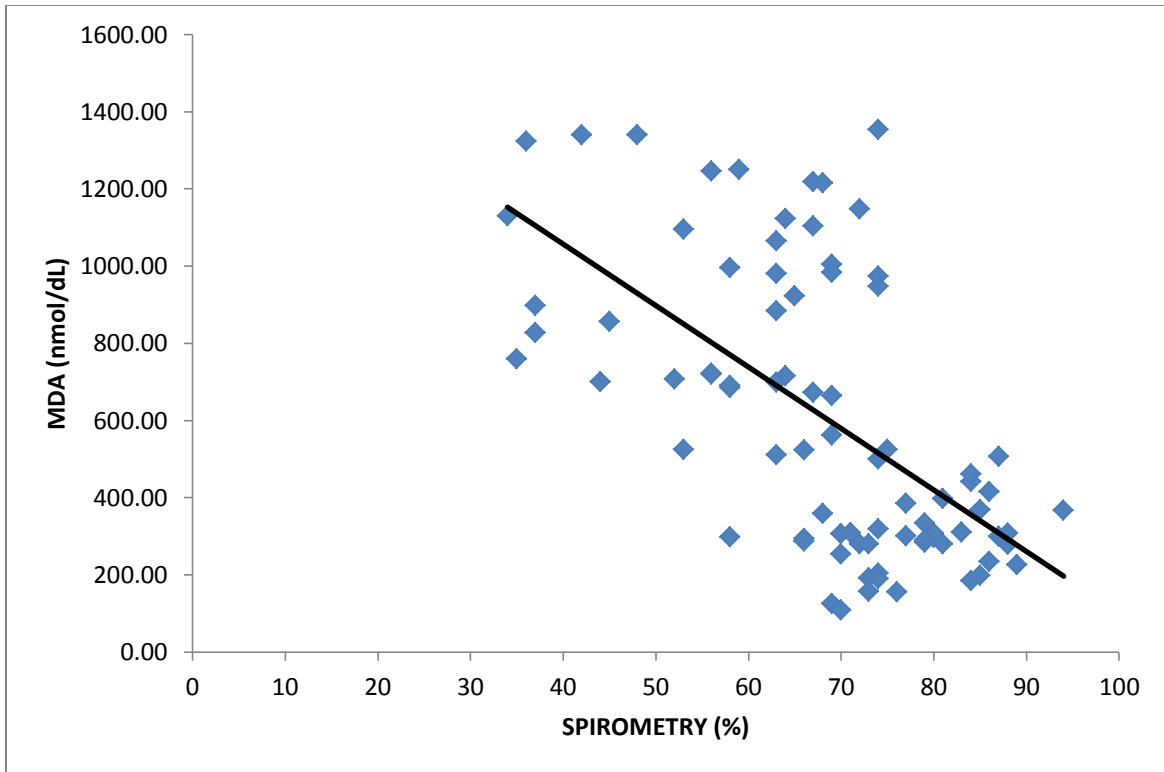
Figure 2:- Scatter plot showing the relation between Malondialdehyde and Spirometry changes in study subjects. (r= -0.604, p-value <0.001).
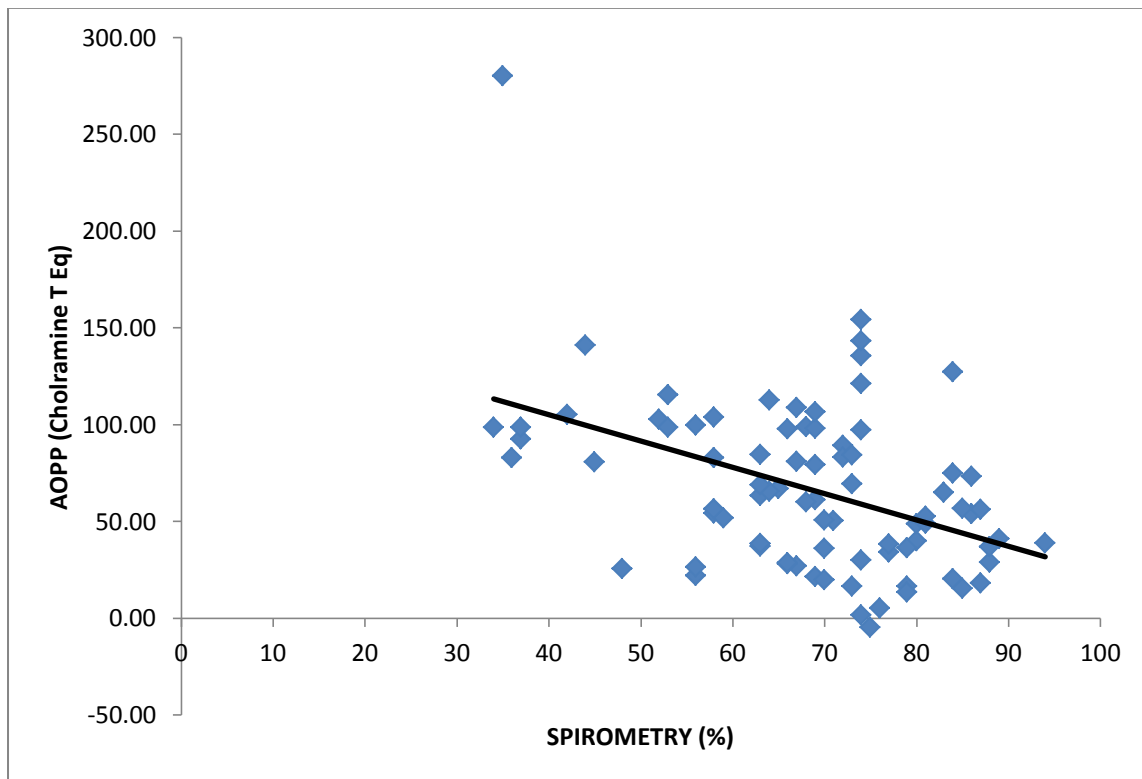

Figure 3:- Scatter plot showing the relation between AOPP and Spirometry changes in study subjects (r= -0.434, p-value <0.001).

Discussion:-

In the present study, oxidative stress parameters and lung capacity changes measured by Spirometric analysis was assessed in smokers and then compared with age-matched healthy non-smokers.

The Adenosine Deaminase (ADA) levels were found to be significantly lower in the smokers (15.40 ±5.67 IU/L) than non-smokers (19.28 ±5.48 IU/L) with the p-value of 0.002 (HS), and it negatively correlated with the smoking history and the number of cigarette consumed by the individual included in the study. Various studies conducted in smokers were found to have increased level of ADA when compared to non-smokers, however a study conducted by Goodarzi (2010), shown in line with our findings. [11] ADA is a polymorphic enzyme that is involved in purine metabolism. The lower level of the ADA in smokers compared to non-smoker normal healthy person can be due to the smoking-induced irreversible cellular damage in the alveoli leading to the reduced mediators and the cellular immunity in the alveolar tissue. This lower level of the ADA in smokers is mainly as the result of decreased ADA activity in them.

MDA which is a marker for lipid peroxidation was increased significantly in smokers (926.61 ±253.57 nmol/dl) when compared to the Non-smokers (301.23 ±100.39 nmol/dl) with the p-value <0.001(HS). This change in the Malondialdehyde (MDA) levels is indicative of lipid peroxidation in the smokers. The lipid peroxidation levels were higher in the smokers in the study conducted by the Ranjbar et al, (2004) and is consistent with the findings in the present study with a significant increase in the level of the MDA in smokers in compared with the Non-smokers.[12,13,14]

The oxidation protein products analyzed in the current study was advanced oxidation protein products (AOPP), which showed statistically significant increased levels in smokers (88.94 ±44.54 chloramine T equivalent) in comparison with the non-smokers (46.26 ±30.07 chloramine T equivalent) with the p-value of <0.001 (HS). A study conducted by the Baskol G (2006), using the AOPP as the marker of the oxidative stress, also showed elevated levels in the diseased group compared to the normal controls.[15] Also study conducted by Zhi PH (2015), oxidative stress products in diabetes mellitus and diabetic retinopathy using the AOPP and MDA as the marker of oxidative stress, found a positive correlation with the oxidative stress showing both as the good marker.[16]

The previous study conducted by Kar K (2014) to access the AOPP as a marker for oxidative stress, has shown a significant difference between the normal individual.[17] A study by Witko-sarsat first time demonstrated the utility of the AOPP as the better marker of the oxidative stress in the patients along with the lipid peroxidation markers.[18] Studies have even demonstrated that AOPP is not only the exquisite marker of oxidative stress, but also an active mediator of the inflammation associated with the uremic state, hence meeting the criteria of a novel marker as uremic toxin.[19]

The Spirometric analysis, required to assess the lung function with respect to the capacity of the expiration of the subjects showed a significant reduction of the FEV1 in the smokers (58.5 ±12.0%) compared to the capacity shown by the non-smokers (77.6 ±7.9%) with the p-value of <0.001 (HS). The Spirometric analysis was used as to assess the lung function according to the guidelines from the GOLD for diagnosing the COPD.[20] Similar kind of study done to assess the lung function test in smokers and nonsmokers has shown a significant reduction in Spirometric results in smokers.[21] A study conducted by James E (2007) found that $FEV_1/FVC\%$ of a normal person is above 70% and smokers will tend to have less than 70%. And that FEV_1 singly can even be analyzed to assess the lung function test and cigarette use was associated with the worsening of all parameters of Spirometry with a reduction in the diffusion capacity, while the total capacity was not greatly affected.[22]

The present study has revealed a clear indication of the increased Oxidative stress levels in the smokers when compared with that of the non-smokers. With elevated levels of MDA and mAOPP in the smokers. The smoke of cigarette being particulate form along with the oxidative stress causing cellular damage to lung tissue, responsible for the lower level of Adenosine Deaminase (ADA) in the smokers.

MDA and AOPP had significant inverse relation with declining performance of lung function due to oxidative damage, and ADA had a weak positive correlation with Spirometric changes.

Cell death induced by cigarette smoke exposure can largely be accounted for by an enhancement in oxidative stress. In fact, the ability of cigarette smoke to generate many reactive oxygen species that cause damage to alveolar

epithelial cells.[23] Apart from oxidative stress, the smokers are even at the risk of reduced lung capacity, which is clearly evident with the decreased in the Spirometry results in smokers which was significantly low when compared with the age-matched Non-smokers.

Conclusion:-

From the current study it is clear that the smokers are at a higher risk of pulmonary diseases& oxidative stress. The oxidative stress caused due to the reactive oxygen species leads to the lipid peroxidation of the cellular membrane and formation of an oxidative product of protein that was assessed by using MDA and AOPP respectively. The utility of the ADA, MDA & AOPP as a marker for the oxidative damage and reduction in lung function test may be a useful tool in educating smokers regarding the ill effect of tobacco use and the possible health derangement and urge them to quit smoking.

Scope:

Study can be extended with the better sample number and the awareness among the smokers can be brought with the evidence of the Spirometric changes in them at early stage. Analysis of total antioxidant levels and the ROS could give a better picture.

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