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Carcino Embryonic Antigen, Cardiac Troponin I and Tumor Necrosis Factor-Alpha Levels in Male Cigarette Smokers in Nnewi Metropolis

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Abstract

Cigarette smoking is known to be associated with the risk of developing certain types of cancers, cardiovascular diseases and chronic obstructive pulmonary diseases. This study was aimed at evaluating the risks of certain types of cancers, cardiovascular diseases and chronic obstructive pulmonary diseases in smokers. A total of 90 subjects were recruited for this study. This comprised 60 male smokers and 30 male non-smokers within the age range of 18 and 65. The levels of carcinoembryonic antigen (CEA), cardiac troponin I (cTnI) and tumor necrosis factor-alpha (TNF-α) were determined using Enzyme-linked Immunosorbent Assay (ELISA) technique. Smokers had significantly higher levels of CEA compared with non smokers P<0.05). However, the average serum level of TNF-α was significantly decreased in the test compared with the control group (P < 0.05). No statistically significant difference was observed in the average serum level of cardiac troponin I in the test subjects compared with that of the control group (P>0.05). However, a strong correlation existed between duration of smoking and the mean levels of CEA (r = 0.296) and cTnI I (r = 0.170) while a negative correlation was observed between the duration of smoking and the mean levels of TNF-α (r = -0.073). The highest serum CEA and cTnI levels were obtained in moderate smokers and the highest serum TNF-α level was observed in heavy smokers. This work suggests that smokers have increased levels of CEA and may regularly face the risk of certain types of cancers.

Keywords: CEA, Troponin I, TNF-α, Smokers.

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INTRODUCTION

During smoking, smoke generated from burnt substance is tasted and absorbed into the blood stream. Usually, the dried leaves of the tobacco plant is rolled into a small paper to create a small, round cylinder called a cigarette. [1] Cigarette smoking serves as a route of administration for recreational drugs. The agricultural product is often mixed with additives and then combusted which releases the active substances. [1] The smoke produced is usually inhaled and the active substances absorbed through the alveoli in the lungs. Cigarette smoke is a complex mixture of more than 4000 different chemicals which could be in the solid phase, gas phase or liquid phase. [2] Some examples of the solid phase chemicals are phenols, nicotine and naphthalene. Carbon monoxide, nitrogen oxides and hydrogen cyanide are the major gases and the liquid vapors include formaldehyde, methane, benzene, ammonia and acetone. [2] The main components of the smoke includes tar, carbon monoxide and nicotine, but they are not alone responsible for the deleterious effects associated with smoking and passive smoking. [2] In view of this chemical complexity, cigarette smoke has multiple highly diverse effects on human health. [3] These substances in cigarette smoke lead to chemical reactions in nerve endings, which increase heart rate and alertness. [4] According to WHO estimates, 5.4 million premature deaths are attributable to tobacco smoking worldwide. [5]

Carcinoembryonic antigen (CEA) is a type of glycoprotein molecule that is produced by cells of the gastrointestinal tract during embryonic development but the production stops before birth. [6] It is expressed in appreciable amounts after birth by carcinomas arising from its site of production. [6] Therefore, CEA is usually present only at very low levels in the blood of healthy adult. CEA can be released by these carcinomas into the circulation causing increased concentrations which may be measured by sensitive radioimmunoassay and similar techniques. [6] It has been shown that small amounts of CEA are

present in the normal adult large intestine and in the circulation of healthy subjects. [6] Inaddition, many epithelial derived tumours at other sites such as cancer of the lungs, breast, pancreas and ovary may also express CEA and be associated with raised circulating blood concentrations. [6] Therefore, the serum levels are over expressed in certain types of cancers especially colorectal carcinomas, which mean that it can be used as a tumour marker in clinical tests. [7]

Troponin consists of three regulatory proteins (troponin C, troponin I, and troponin T) that controls muscle contraction in skeletal muscle and cardiac muscle, but not smooth muscle. [8] Troponin acts with intracellular calcium to control the interaction of actin and myosin filaments in striated muscles . [8] Three isoforms exist for troponin I (TnI). Two are present in skeletal muscle which are the fast skeletal troponin I(fsTnI) and the slow skeletal troponin I(ssTnI), the third is present only in the cardiac muscle which is the cardiac troponin I (cTnI). Damage to cardiac myocytes results in loss of membrane integrity which causes the release of cTnI into the circulation. [9]

Tumor necrosis factor-alpha (TNF-α) is a pro-inflammatory cytokine incriminated in multiple inflammatory diseases. [10] TNF-α was originally described as a factor produced by the endotoxin stimulated haemorrhagic necrosis of tumors. [11] It is a srong pro-inflammatory cytokine with pleiotropic properties and a key mediator of inflammation. [12] Different studies have shown that TNF-α and its receptors could be useful biomarkers with a potential prognostic value in several diseases associated with local and systemic inflammation. [12] Important evidence also suggests that cigarette smokers are at higher risk of inflammatory cardiovascular disease when compared to non-smokers. [13] It has been shown that TNF-α production in the blood from smokers exposed to endotoxin was 38% higher than in non-smokers. [14] This study determined the levels of carcinoembryonic antigen (CEA), cardiac troponin I (cTnI) and tumor necrosis factor-alpha (TNF-α) inorder to evaluate the risks of certain types of cancers, cardiovascular diseases and chronic obstructive pulmonary diseases in smokers.

Statement of Problem

Cigarette smoking remains a major preventable public health problem associated with premature deaths worldwide. Annually, Nigerians spend about N89bn on tobacco with South-east topping smoking league survey. [15] The prevalence of smoking among adolescent in Calabar city, southern Nigeria in 2012 was 6.4% with the prevalence rate being higher among males. [16] Also the prevalence of smoking among adolescents in Port Harcourt, south-eastern Nigeria presently is 7.1% with a prevalence rate higher in males than in females. [17] The prevalence rate among adults obtained from the national survey carried out in 2002 was 8.6%. [18] Between 2012 and 2014, the prevalence of smoking in Nigeria was 10%. [19] This shows that the prevalence of smoking is gradually on the increase. The south-easterners are the major consumers of tobacco in Nigeria. [15] Moreover, cigarette smoking is a significant risk factor for certain types of cancers and atherosclerosis which over time can lead to chest pain and myocardial infarction. [20] It is the major cause of chronic obstructive pulmonary disease development. [21] Information regarding the relationship of cigarette smoking with certain types of cancers, cardiovascular diseases and chronic obstructive pulmonary diseases is scanty especially in sub-Saharan Africa. This study determined the levels of carcinoembryonic antigen (CEA), cardiac troponin I (cTnI) and tumor necrosis factor-alpha (TNF-α) inorder to evaluate the risks of certain types of cancers, cardiovascular diseases and chronic obstructive pulmonary diseases in smokers.

Justification of the Study

Cigarette smoking is one of the major causes of preventable deaths globally. It is a significant risk factor in certain types of cancers and heart diseases. [2] Data generated from the present study may provide information on selection of appropriate biomarkers for predicting the onset of conditions associated with cigarette smoking such as certain types of cancers, cardiovascular diseases and chronic obstructive pulmonary diseases. The study may also provide invaluable information on the prognostic evaluation of these conditions.

Aim

This study is aimed at evaluating the risks of certain types of cancers, cardiovascular diseases and chronic obstructive pulmonary diseases in smokers.

Specific Objectives

1. To determine the levels of CEA, cTnI and TNF-alpha in adult male smokers and male non smokers
2. To correlate the levels of these parameters with the duration of smoking.
3. To evaluate the levels of these parameters in the different age ranges.
4. To determine the levels of these parameters in light, moderate and heavy smokers.

MATERIALS AND METHODS

Inclusion Criteria: Adult male smokers between the ages of 18 and 65, adult male non-smokers of matching ages.

Exclusion Criteria: subjects outside the age bracket, subjects with a previous history of cancer, cardiovascular disease or Chronic obstructive pulmonary disease.

Materials

ELISA machine (Fx 2400 Awareness technology, Germany).

Methods

Research Design

This is a cross sectional study designed to evaluate the levels of CEA, cTnI and TNF-α in adult male cigarette smokers in Nnewi metropolis, Anambara State, Nigeria.

A total of 90 subjects were recruited using the convenient sampling technique. It comprised 60 adult male smokers and 30 adult male nonsmokers within the age range of 18 and 65. The age of the smokers and duration of smoking were determined using a standard questionnaire.

Study Site

This study was performed at the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra state, Nigeria.

Sample size

The sample size (N) was determined according to the method of [22]

Using the formula : $N = Z2\ PQ\ /d2$. [22]

Where N= minimum sample size

Z= standard deviation at 95% confidence interval which is 1.96

P= Prevalence rate (which is 4%= 4/100 = 0.04)

Q= Alternate proportion given as (1 - P)= 1 - 0.04 = 0.96

d= degree of precision which is 0.05.

The prevalence of smoking among adults in Nigeria is 4%. [23]

Therefore N= (1.96)2 x 0.04 x 0.96 / (0.05)2 = 59

Therefore the sample size used for this research work is 60 samples.

Sample Collection

About five mililitres of blood was collected from study participants and dispensed into plain tubes and susequently allowed to clot. The samples were then centrifuged at 5000rpm for 5 minutes. Serum was separated and stored at -20°C.

Estimation of Carcinoembryonic Antigen

The estimation of CEA was determined according to the methods described by [24]. This method is basically an Enzyme Linked Immunosorbent Assay (ELISA) procedure.

Procedure:

The required number of coated wells were secured in the holder, then 50µL of the standard and specimen were added into appropriate wells and 100 µL of Enzyme Conjugate Reagent was equally added into each well, thoroughly mixed for 30 seconds and incubated at room temperature for 60 minutes. After incubation the mixture was removed by emptying plate content into a waste container. The wells were then rinsed and emptied 5 times with distilled water. The wells were sharply stroke onto absorbent paper to remove all residual water droplets. Then 100 µL of TMB Reagent was dispensed into each well and gently mixed for 10 seconds, Incubation was performed at room temperature for 20 minutes. The reaction was stopped by adding 100µL of Stop Solution to each well and gently mixed for 30 seconds. It was read at an optical density of 450 nm within 15 minutes.

Estimation of Cardiac Troponin I

cTnI levels were estimated according to the methods described by [25] This is a solid phase enzyme-linked immunesorbent assay method.

Procedure:

First, the required numbers of coated wells were secured in the holder. Then 100 µL of cTnI HRP Conjugate was dispensed into each well. About 100 µL of calibrators and diluted samples were dispensed into the appropriate wells. The setup was thoroughly mixed and incubated at room temperature (18-25°C) for one hour. The incubated mixture was discarded and the microtitre wells were washed 6 times with the washing solution that was provided. The wells were stroke sharply onto an adsorbent paper to remove all residual droplets. Then 100 µL of TMB reagent solution was dispensed into each well and incubated at room temperature for 20 mins. Finally, 100 µL of Stop Solution was added into each well to stop the reaction. It was mixed gently till all the blue color changed to yellow. It was Read at an absorbance of 450 nm.

Estimation of Tumor Necrosis Factor-Alpha

TNF alpha levels were determined according to the methods described by [26] This procedure is a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) method.

Procedure:

The needed number of coated wells were secured in the holder, Then 100 µL of the standard and sample were added to the appropriate microtiter wells and incubated for 2 hours at room temperature. The incubated mixture was decanted from the wells and the wells were washed 4 times using the assay buffer provided. Then 100 µL of biotinylated anti-TNF-α (Biotin Conjugate) solution were pipetted into each well except the chromogen blank and incubated for 1 hour at room temperature. The solutions were decanted from the wells and washed 4 times using the assay buffer provided. Subsequently, 100 µL of Streptavidin-HRP Solution was added to each well except the chromogen blank and the mixture was incubated for 30 minutes at room temperature. The solution was decanted from the wells and washed 4 times. About 100µLof substrate solution was added to each well. It was Incubated for 30 minutes at room temperature. Finally, 100 µL of the Stop Solution was added to each well and the absorbance of each well was read at 450 nm within 2 hours.

Statistical Analysis

The data collected was analyzed statistically using the students t-test and the analysis of variance (ANOVA). Values were deemed significant if p is less than 0.05. Correlation of parameters were elucidated using the Pearson's correlation coefficient.

RESULT

In table 1, there was a statistically significant increase in the mean serum levels of Carcinoembryonic Antigen in smokers compared with non-smokers (P<0.05). However, the average serum levels of Tumor Necrosis Factor-Alpha decreased reasonably in the test compared with the control group (P < 0.05) while no significant difference was observed in the mean serum level of cardiac troponin I in the test subjects compared with that of the control group (P>0.05).

Table 1: Levels of carcinoembryonic antigen, cardiac troponin I and tumor necrosis factor alpha in smokers and non-smokers

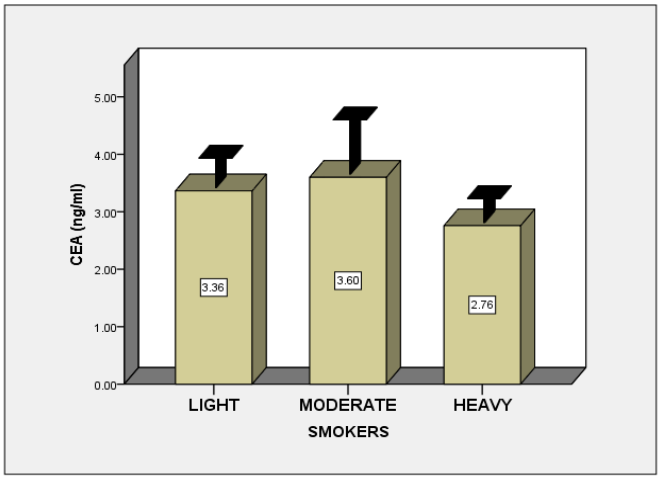
Parameters	Smokers N= 60 (Mean ± SD)	Non-smokers N= 30 (Mean ± SD)	t-test	p-value
CEA (ng/ml)	3.30 ± 1.66	1.94±0.83	3.424	0.000
Cardiac Troponin I (ng/ml)	0.96 ± 0.36	0.84 ± 0.15	1.934	0.056
TNF-α (pg/ml)	167.53±43.65	209.05±84.64	-2.298	0.024

Findings from table 2 showed that a positive correlation existed between duration of smoking and the average serum levels of carcinoembryonic antigen (r = 0.296) and cardiac troponin I (r = 0.170) while a negative correlation was observed between the duration of smoking and the average serum levels of TNF-a (r = -0.073).

Table 2: Correlation of Carcinoembryonic antigen, cardiac troponin I and Tumor necrosis factor-alpha with duration of smoking

Groups (n=60)	r	p-value
Duration of smoking vs CEA	0.296	0.037
Duration of smoking vs Troponin I	0.170	0.237
Duration of smoking vs TNF α	-0.073	0.046

Figure 1 shows that there was no statistically significant difference between the mean serum values of carcinoembryonic antigen in light, moderate and heavy smokers (P>0.05). The highest CEA level was obtained in moderate smokers.



Error Bars: +/- 2 SE

Figure 1: Levels of carcinoembryonic antigen in light, moderate and heavy smokers

In figure 2, there was no significant difference between the average serum values of Cardiac Troponin I in light, moderate and heavy smokers (P>0.05). The highest cTnI level was observed in moderate smokers.

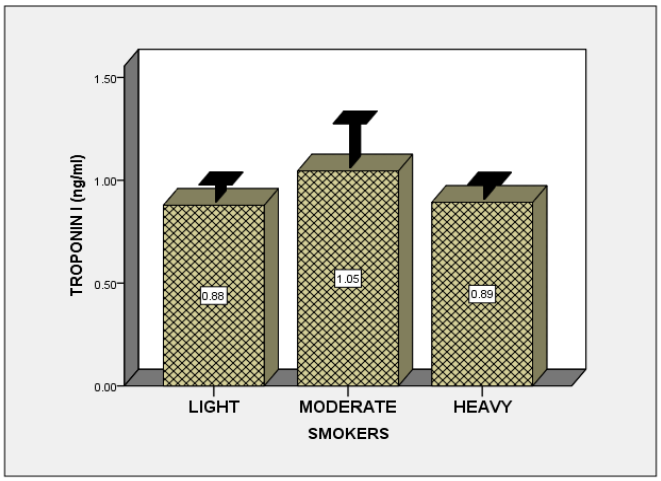


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Figure 2: Levels of cardiac troponin I in light, moderate and heavy smokers

It was noted in figure 3 that there was a significant difference between the average serum values of Tumor Necrosis Factor-alpha in light, moderate and heavy smokers (P<0.05). The highest TNF-a level was obtained in heavy smokers.

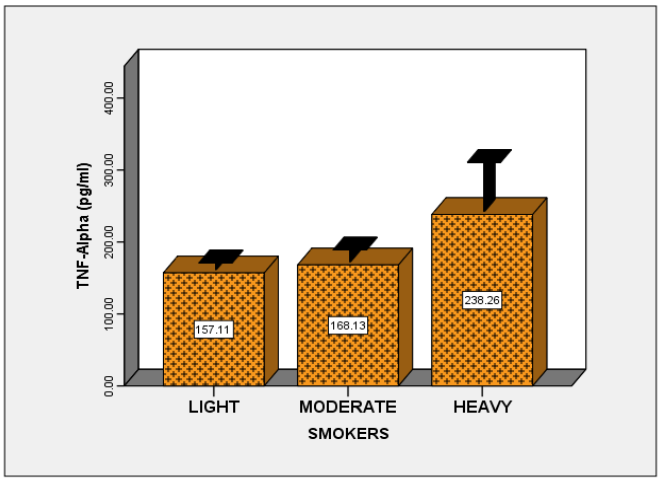

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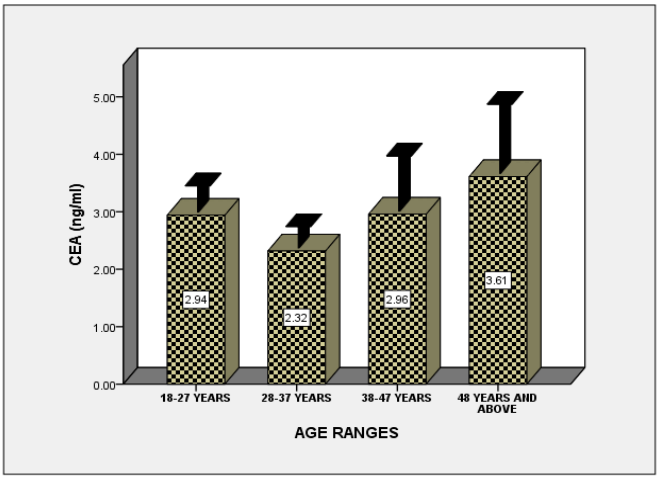
Figure 3: Levels of carcinoembryonic antigen in light, moderate and heavy smokers

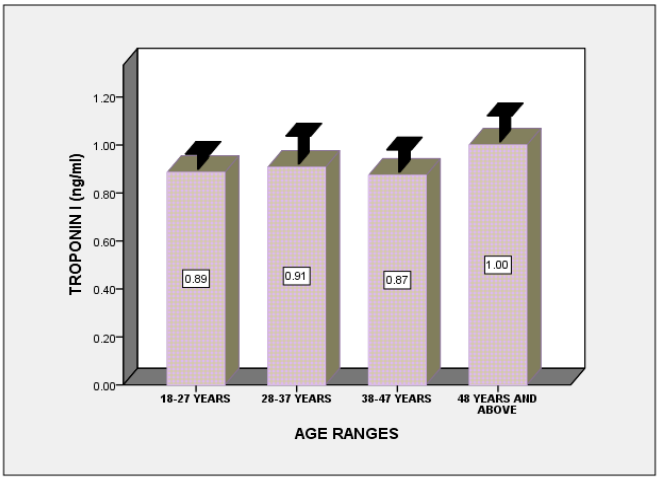
Results in figure 4 showed that there was no significant difference between the mean serum values of carcinoembryonic antigen in smokers of different age ranges (P>0.05). The highest CEA value was obtained in age range of 48 and above.



Error Bars: +/- 2 SE

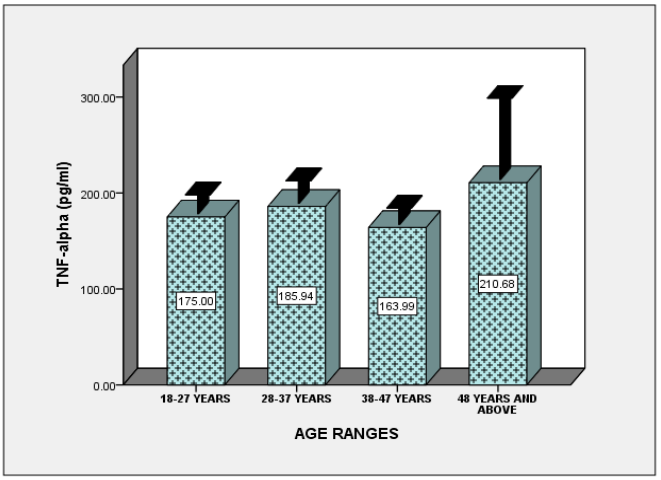
Figure 4: Levels of carcinoembryonic antigen in different age ranges

There was no significant difference between the average serum values of cardiac troponin I (figure 5) in smokers of different age ranges (P>0.05). The highest cardiac troponin I level occurred within the age range of 48 and above.



Error Bars: +/- 2 SE

Figure 5: Levels of Cardiac Troponin I across the different age ranges



Error Bars: +/- 2 SE

Figure 6: Levels of tumor necrosis factor alpha across different age ranges

There was no significant difference between the average serum values of Tumor Necrosis Factor-alpha (figure 6) in smokers of different age ranges (P>0.05). The highest TNF-a value was obtained in age range of 48 and above.

DISCUSSION

In this study, there was a significant increase in the mean serum level of Carcinoembryonic Antigen in smokers compared with non smokers. (P<0.05). This is in line with the work done by [27] who reported that in male subjects, serum CEA levels were significantly higher in smokers than in nonsmokers, females however, had no significant difference between smokers and nonsmokers. In this study, no significant difference was noted in the average serum level of cardiac troponin I in the study participants compared with that of the control group (P> 0.05). This is in agreement with the study done by, [28] who showed that Smoking cessation showed no significant decrease in mortality in patients with cardiovascular diseases. Results from this study showed that the mean serum level of Tumor Necrosis Factor-alpha was significantly decreased in the test compared with the control group (P<0.05). This is in agreement with the work done by, [29] who compared concentrations of tumor necrosis factor, interleukin (IL)-6, and IL-8 in bronchoalveolar lavage fluid (BALF) and estimated the capacity of BALF macrophages to release TNF and IL-6 in vitro in smokers and non-smokers, they found that cells from smokers released less TNF-α compared to nonsmokers. Factors which have a protective effect on smokers could also be responsible for the lower levels of TNF-α in smokers observed in our study, suggesting that smoking could also have a protective effect on airway inflammation. Since most scientific studies report the effect of body weight or the percentage of body fat as the most important factors influencing the levels of TNF-α, [30] [31] [32], It is likely that the higher TNF-a observed in the control group (non-smokers) resulted from adipose tissues since the body weight or the percentage of body fat of the subjects were not put into consideration in our study.

A positive correlation existed between length of time of smoking and the amount of carcinoembryonic antigen (r= 0.296). This is in line with the study done by, [33] and [34] they reported that smoking and its duration effects CEA levels. Heavy smoking over many years raises blood CEA levels. [34] In smokers, the most important parameter that affects cancer risk is the duration of regular smoking. [35] Duration is determined by the age at commencement and attained age in current smokers. [35] There was a positive correlation between duration of smoking and the levels of cardiac troponin I (0.170). A negative correlation was observed between the duration of smoking and the levels of TNF-a (r -0.073). Nicotine at low concentrations was shown to stimulate TNF-α secretion, [36] but at levels equivalent to that in the plasma of smokers it inhibited TNF -α and IL-2 production. [37] A prolonged exposure to nicotine increases its concentration and thereby inhibits TNF-a production. This explains the negative correlation between the duration of smoking and TNF-a levels observed in our study.

However, there was a significant difference between the average serum values of Tumor Necrosis Factor-alpha in light, moderate and heavy smokers (P<0.05). The highest TNF-α level was observed in heavy smokers. The mean TNF-a value of heavy smokers was significantly higher than the mean value of moderate and light smokers, and the mean TNF-α value of light smokers was significantly lower than that of moderate smokers. This implies that the expression of tumor necrosis factor alpha increases with the number of cigarettes smoked per day, revealing that the effect of smoking on TNF-α is dose dependent because the highest levels were observed in heavy smokers while the lowest value was observed in light smokers. The contents of a cigarette smoke may influence both the production of cytokines and acute-phase proteins and the antioxidant defenses by exerting its chronic inflammatory stimulus on the macrophage-monocyte system. [38] This finding in our work is consistent with the study of [39]. They reported that smoking and microvascular complications exert an additive and deleterious effect on sTNF-R1 levels; the effect of smoking is strictly dose dependent. Similar results were reported by [40] .

CONCLUSION

This study has shown that there was a significant increase in the mean serum level of Carcinoembryonic antigen in smokers compared with non-smokers. Our work has also shown that a positive correlation existed between duration of smoking and the mean serum levels of carcinoembryonic antigen and cardiac troponin I while a negative correlation was observed between the duration of smoking and the mean serum levels of TNF-a. The highest serum CEA and cTnI levels were obtained in moderate smokers. However, there was a significant difference between the mean serum values of tumor necrosis factor-alpha in light, moderate and heavy smokers. The highest serum TNF-a level was obtained in heavy smokers. The highest serum CEA, cTnI and TNF-a values were obtained in age range of 48 and above.

Conflict of interest

The authors of this article declare that there is no conflict of interest.

Funding

Funding for this research was from personal sources.

Ethical Consideration

Ethical approval was obtained from ethics committee of the Nnamdi Azikiwe University Teaching hospital Nnewi (NAUTH).

Informed Consent

Consent was sought and obtained from all participants prior to this study.

Author's contribution

M.P.O., E.K., C.G.O. designed research, E.K., C.O.M., A.J.C., A.J.C., performed research, I.N.C., C.G.O., analysed data, C.G.O., O.E.C., M.P.O., wrote paper.

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