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Biochemical effects of Cigarettes Smoking on Liver functions of Sudanese diabetics smokers

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Abstract

The present study was conducted in Khartoum state (Sudan) as a cross-sectional study in 200 participants (100 smokers and 100 non-smokers), to investigate the effects of Cigarettes Smoking on Liver functions of Sudanese smokers. The smokers were categorized into either current smokers (70 participants) or past smokers (30 Participants). Parameters were analyzed by using spectrophotometer. From the results, the levels of liver function tests (TB, Albumin and globulin) were significantly higher in non-smokers (6.0, 3.6 and 2.43 g/dl) as compared to current smokers (4.4, 2.4 and 1.98 g/dl) and past smokers (4.7, 2.7 and 1.93 g/dl). Meanwhile, ALT was significantly increased in current and past smokers as compared to non-smokers, where as AST was significantly higher in current smokers as compared to non-smokers, while there was no significant difference between current and past smokers as well as between past and non-smokers for this parameter. In addition, heavy and long duration of smoking were both associated with low total protein, albumin and AST levels .Also liver function tests were not significantly differing in current smoker's non-diabetics and non-smokers diabetics except total protein. From the results when, liver enzymes raised that may lead to increase proteins metabolism and increase blood glucose. These signs may place cigarettes smoking one of the top causes of hepatitis, pancreatitis, insulin resistance and finally diabetes mellitus type II. On the other hand, smoking cessation in diabetics lead to decrease the risk of hepatitis, Also regular physical activity and using medication regularly in diabetics lead to the same actions above; in addition diabetes complications were decreased.

Keywords: Cigarettes Smoking, Liver functions, diabetic's smokers

INTRODUCTION:

Smoking is one of the leading causes of preventable death globally. In the United States, about 500,000 deaths per year are attributed to smoking-related diseases and a recent study estimated that as much as 1/3 of Chinese male population will have significantly shortened life spans due to smoking. Male and female smokers lose an average of 13.2 and 14.5 years of life, respectively. At least half of all lifelong smokers die earlier as a result of smoking.(Sander *etal*.,2009, Matthew Hilton.,2006)

There are many ingredients in tobacco smoke that cause many diseases in the body including, infection, cancers and heart disease. Metha *et al,* (2008) and Zhong *et al* (2008) and every 6 minutes one person die.

In the world due to smoking Mathers CD and loncar 2006. Cigarette contains over 4,000 chemicals, 200 of them are poisons

,contains over 80 cause cancer include

(CO, free radicals, nicotine, and tar)Abel *et al* (2005) and Carel *et al* (1985). Most dangerous component are tar and Carbon monoxide. Tar contains chemicals carcinogens which deposits in lung. Also smoke produce CO which binds to Hb retard carrying of oxygen and lead to hypoxia , lung cancer, kidney cancer, heart disease and stroke Payam *et al* (2005) . Cigarette smoke affects many organs. The liver is one of those Organs that might be affected by smoking despite the fact that there is

no direct contact between liver and smoking Sanger *et al,* (2008). Liver is vital organ of vertebrates play many roles in the body such as: metabolism with numerous function including: regulation of glycogen storage, de composition of RBCS, plasma protein synthesis, hormone production and eliminate toxins from the body .To assess the liver function there are many tests which can be conducted inside clinical laboratory. Some of these tests are, serum total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin Kalaed *et al,* (2014).The determination functions of liver must be done carefully and precise because these parameters can affected by external factor such as: environmental factor . Some recent studies were conducted on effect of cigarette smoking on albumin, liver enzymes (AST, ALT and ALP), and bilirubin in males' smoker.Many studies showed that effect of cigarette smoked in LFT caused significant increase in (AST, ALT and ALP)also significant decrease in serum albumin and serum total bilirubin Nathwani *et al,* (2005) According to the American Heart Association about 22% of adults with diabetes smoke, even though U.S. research indicates that the most harmful effect of smoking is linked to a significantly higher risk of developing Type 2 diabetes. In fact, the University of Lausanne (Lausanne, Switzerland) analyzed several studies involving more than one million patients and discovered that one of the risks of smoking is a 44% higher chance of developing Type 2 diabetes compared with non-smokers. In addition, the risk increases with the average number of cigarettes smoked daily. Smoking has been identified as a possible risk factor for insulin resistance (a precursor for diabetes). Also been shown to deteriorate glucose and lipid metabolism which may lead to the onset of type 2 diabetes.(Fagard, 2009) There is also some evidence which suggests that

smoking increases diabetes risk through a body mass index independent mechanism.(Cullen *et al*, 2009, Nagaya,2008)

Numerous cross-sectional studies indicate that body weight, or body mass index (BMI; in kg/m2), is lower in cigarette smokers than in nonsmokers. In the World Health Organization Monitoring Cardiac Disease surveys, BMI was lower in smokers than in nonsmokers in 20 (men) and 30 (women) of the 42 populations, and there was no population in which smokers had a higher BMI than did nonsmokers.(Molarius *et al*,1997)

Smoking's effect on body weight could lead to weight loss by increasing the metabolic rate, decreasing metabolic efficiency, or decreasing caloric absorption (reduction in appetite), all of which are associated with tobacco use. The metabolic effect of smoking could explain the lower body weight found in smokers. (Dallosso *et al*,1984)Few studies have evaluated the chronic metabolic effects of smoking, and the results have conflicted. After 30 day of smoking cessation, the resting metabolic rate in female quitters was shown to be 16% lower than it had been when they were smoking, and an increase in body weight was attributable to a decrease in resting metabolic rate and an increase in caloric intake.(Moffatt,1991)

Physical activity increases metabolic rate and may help to control body weight, but smokers tend to be less physically active than nonsmokers.(Klesges RC *et al*, 1990)

Moreover, those studies have been reported primarily from Western and Asian countries, while the study of the effects of the cigarette smoking on Liver function tests for persons living in African countries especially in Sudan have had limited study. Thus, this study was conducted in a Sudanese population to evaluate the biochemical effects of cigarettes smoking on Liver functions of Sudanese smokers. Also to know if cigarettes smoking can increases the risk of hepatitis and Diabetes complications.

OBJECTIVES:

1. To examine the effects of cigarettes smoking on Liver functions of Sudanese diabetics smokers.
2. Also in this study, we examined the association between smoking and hepatitis including cigarette smoking as a risk factor, how smoking can lead to multiple complications of diabetes and the benefit of smoking cessation.

Materials & Methods:

Design of the study:

This study was conducted at the department of Biotechnology ,faculty of Science &Technology - Omdurman Islamic University for a period of 2.5 years (2013 to 2015).Among 100 Sudanese voluntary cigarette smokers (current &past) and 100 non-smokers , their age range between 20-80 years. Two groups (smokers and non-smokers) were collected with the same range of age for statistical comparison. The smokers were regularly consuming minimum of 3 cigarettes per day for at least 3 years.The clinical data, medical history and other relevant information were collected from subjects by personal interview through the designed study questionnaire.

Samples and data collection:

Under a septic condition, about 4 ml of venous blood were collected from each volunteer by vein puncture (after overnight

fasting 10-12 hours) and placed in heparinized containers, and allowed to clot then centrifuged at 3000 rpm for 5 minutes to obtain plasma which kept in epindorfe tubes in refrigerator at 2-8 c^0.

In questionnaires, each respondent was asked about smoking behavior. We defined subjects as non-smokers (those whom never smoked cigarettes in their lifetime), current smokers (those who smoking now), and past smokers (those whom smoked at least 3 cigarettes for 3years in the past but did not smoke now). Current smokers were asked to provide information about cigarettes consumption, which categorized according to no. of cigarettes smoked per day to (3-13, 14–24 and >24). Alsosmoking duration per years categorized according tothe following ranges (3-13, 14–24 and >24).

Liver function tests:

Plasma total protein, albumin, ALT and AST, were estimated in all subjects by a kit methods on spectrophotometer. (Biosystems S.A.**,**2011**)** Commercially available test kits, products of BioMed Biotechnologies, Egyptian were used and with the manufacturer's instructions strictly adhered to. Plasma globulin was determined as the difference between total protein and albumin.

Results:

Table (1) shows the minimum , maximum and mean ±SD for all studied biochemical parameters in each smoking categories (quantitative data) , whereas table (2), shows categorical data (qualitative data) for the habits or variables that associated with the smokers (Age, sex, diabetes status and complications)

Table (1) Descriptive statistics shows the Minimum, Maximum, and Mean ±SD for quantitative variables

Table (1) Descriptive statistics shows the Minimum, Maximum, and Mean ±SD for quantitative variables

Variables	Current smokers (N=70)			Past smokers N=30)			Non- Smokers (N=100)		
	Minimum	Maximum	Mean±SD	Minimum	Maximum	Mean±SD	Minimum	Maximum	Mean±SD
Age /Year	21	80	54.7±18.1	22	75	48.4±18.3	20	80	48.3±18.7
T.protein(g/dl)	2.3	7.3	4.4±1.2	2.3	7.5	4.7±1.5	3.4	8.2	6.0±1.1
Albumin(g/dl)	0.7	5.6	2.4±0.9	1.0	5.7	2.7±1.4	1.4	5.5	3.6±0.9
Globulin(mg/dl)	0.9	3.5	2.0±0 .6	1.0	3.1	1.9±0.5	0.3	4.6	2.4±0.9
ALT(IU/L)	23.0	53	36.9±5.3	29.9	56.3	37.9±5.4	6.0	43.2	29.4±7.2
AST(IU/L)	17.9	47	31.1±8.2	17.6	43.2	28.8±6.8	15.9	44	27.9±7.3

Table (2) Descriptive statistics shows the frequency and percentage for sex diabetes status and diabetes complication of participants.

Cases		Current smokers		Past smokers		Non- Smokers		
		Frequency	Percentage	Frequency	Percentage	Frequency	Percentage	Total
Sex	Male	58	29	27	13.5	84	42	169
	Female	12	6	3	1.5	16	8	31
	Total	70	35	30	1	100	50	200
Diabetic Status	Diabetics	35	17.5	15	7.5	50	25	100
	Non-diabetics	35	17.5	15	7.5	50	25	100
	Total	70	35	30	15	100	50	200
Diabetes complications	Foot problems	5	14.3	2	13.3	3	6	6
	Amputation	3	8.6	1	6.7	0	0	0
	Neuropathy	6	17.1	4	26.7	1	2	2
	Retinopathy	7	20	3	20	4	8	8
	Nephropathy	3	8.6	0	0	1	2	2
	None	11	31.4	5	33.3	41	82	82
	Total	35	100	15	100	50	100	100

Effects of smoking status on liver function tests of smokers and non-smokers:

Table (3&4) show that TB, Albumin and globulin were significantly higher in non-smoker (6.0, 3.6 and 2.43 g/dl) as compared to current smoker (4.4, 2.4 and 1.98 g/dl) and past smoker (4.7, 2.7 and 1.93 g/dl).

On the other hand, there were no significant differences between Current and past smokers for these parameters. Meanwhile, ALT was significantly increased in current and past smokers as compared to non- smokers, where as AST was significantly higher in current smoker as compared to non-smokers, while there was no significant difference between current and past smokers as well as between past and non- smokers for this parameter.

Levels of total protein, albumin and globulin as shown in table (3&4) , were significantly higher in non-diabetics subject as compared to diabetics, while the reverse was true for ALT and AST.

Table (3) shows the effects of smoking status on Liver function test of smokers and non-smoker.

	Total protein(g/dl)			Albumin(g/dl)			Globulin(g/dl)			ALT (IU/L)			AST(IU/L)		
	Diabetics	None	Mean	Diabetics	None	Mean	Diabetics	None	Mean	Diabetics	None	Mean	Diabetics	None	Mean
Current Smokers	3.9^{d}	4.9^{c}	4.4^{b}	2.0^{c}	2.7^{b}	2.4^{c}	1.7^{c}	2.2^{b}	1.98^{b}	38.7^{ab}	35.1^{c}	36.9^{a}	34.4^{a}	27.7^{b}	31.0^{a}
Past-Smokers	3.7^{d}	5.7^{b}	4.7^{b}	1.8^{c}	3.7^{a}	2.7^{b}	1.8^{c}	2.0^{bc}	1.93^{b}	39.5^{a}	36.4^{bc}	37.9^{a}	29.5^{b}	28.2^{b}	28.8^{ab}
Non-Smokers	5.4^{b}	6.6^{a}	6.0^{a}	3.4^{a}	3.8^{a}	3.6^{a}	2.0^{cb}	2.8^{a}	2.4^{a}	34.7^{c}	24.8^{d}	29.7^{b}	29.2^{b}	26.6^{b}	27.9^{ab}
Mean	4.3^{b}	5.7^{a}		2.4^{b}	3.4^{a}		1.9^{b}	2.4^{a}		37.6^{a}	32.1^{b}		31.0^{a}	27.5^{b}	
S.E ±of S.Status			0.17			0.15			0.13			0.86			1.3
S.E ±of D.Status			0.10			0.09			0.07			0.49			0.7
S.E ±of Interaction			0.25			0.21			0.17			1.21			1.2

*Means which have similar letters are not significantly different at 0.05 level of probability according to DNMRT.

Table (4) Mean Square values showed the effect of Smoking Status & No. of cigarettes on Liver functions of smokers.

Source	d.f	T.protein(g/dl)	Albumin (IU/L)	Globulin(g/dl)	ALT(IU/L)	AST(IU/L)
Smoking status	3	10.9**	7.8**	1.3**	27.2^{ns}	166.8*
No. of cigarettes/day	2	2.4^{ns}	1.7^{ns}	0.3^{ns}	20.4^{ns}	70.3^{ns}
Interaction	2	3.2*	0.8^{ns}	1.8**	52.7^{ns}	12.9^{ns}
Error	92	1	0.81	0.3	28.1	54.3
C.V %		2.2	36	25.5	14.2	24.2

n.s=No significant effects

*=Significant at 0.05 level of probability

**=Significant at 0.01 level of probability

Correlation between (age, smoking duration and number of cigarettes/day) and Liver function tests of smokers:

Table (5) Shows that ,age, smoking duration and number of cigarettes per day for smokers reflected (+)ve, medium to weak and significant correlation with AST , but there were (+)ve , weak and insignificant correlation with ALT, while they showed (–)ve, weak and insignificant correlation with total protein ,Albumin and globulin, with an exception that smoking duration showed no significant correlation with AST.

Table (5) shows Correlation between (age, smoking duration and number of cigarettes/day) and Liver function tests of smokers:

Variables	r&P	TB (g/dl)	Alb(IU/L)	Glob(g/dl)	ALT(IU/L)	AST(IU/L)
Age	r	-0.437^{**}	-0.360^{**}	-0.274^{**}	0.028^{ns}	0.284^{**}
	p	0.000	0.000	0.006	0.783	0.004
Duration	r	-0.506^{**}	-0.385^{**}	-0.356^{**}	0.088^{ns}	0.105^{ns}
	p	0.000	0.000	0.000	0.382	0.279
No. of cigarettes/d	r	-0.385^{**}	-0.352^{**}	-0.148^{ns}	0.008^{ns}	0.276^{**}
	p	0.000	0.000	0.142	-0.938	0.005

Relationship (regression) between (smoking duration and number of cigarettes/day), and liver function tests of smokers:

Table (6) shows that there was (–)ve and significant ($p<0.01$), (d.f=98) relationship between smoking duration and no. of cigarettes per day in one side and smokers total protein , albumin and globulin. Whereas the relationship between these variables and both ALT and AST was (+)ve and in significant , except for no. of cigarettes /day with AST ,in which there was significant ($p<0.01$) relationship.

The B.value indicates that , for each one unit change in the independent variable (no. of cigarettes/day), the dependent variables Total protein, Albumin ,Globulin , ALT and AST will be changed by (-0.03) g/dl, (-0.02) g/dl , (-0.01) g/dl , (-0.01) IU/L and (0.12) IU/L , respectively.

Meanwhile, changing of one year duration of smoking, resulted in change for the above dependent variables by (-0.09) g/dl, (-0.06) g/dl, (-0.03) g/dl, (0.07) IU/L and (0.12) IU/L, respectively.

Table (6) shows the relationship (regression) between age , smoking duration and number of cigarettes/day, and liver function tests of smokers:

Variables	B.value	d.f	S.E±	p.value	Level of cig.
Total protein(g/dl)					
Age(year)	-0.03	98	0.01	0.000	**
Smoking duration(year)	-0.09	98	0.02	0.000	**
No. of cigarettes /day	-0.10	98	0.02	0.000	**
Albumin(g/dl)					
Age(year)	-0.02	98	0.01	0.000	**
Smoking duration(year)	-0.06	98	0.01	0.000	**
No. of cigarettes /day	-0.08	98	0.02	0.000	**
Globulin(g/dl)					
Age(year)	-0.01	98	0.003	0.006	**
Smoking duration(year)	-0.03	98	0.01	0.000	**
No. of cigarettes /day	-0.02	98	0.01	0.142	ns
ALT(IU/L)					
Age(year)	-0.01	98	0.03	0.783	ns
Smoking duration(year)	-0.07	98	0.08	0.382	ns
No. of cigarettes /day	-0.01	98	0.011	0.938	ns
AST(IU/L)					
Age(year)	-0.12	98	0.04	0.004	**
Smoking duration(year)	-0.12	98	0.12	0.297	ns
No. of cigarettes /day	-0.45	98	0.16	0.005	**

n.s=No significant effects

*=Significant at 0.05 level of probability

**=Significant at 0.01 level of probability

Discussion

The present study described a detailed effect of cigarettes smoking on the Liver function tests (LFTs) .In which (TB, Albumin and globulin) were significantly higher in non-smoker as compared to current smoker (4.4, 2.4 and 1.98 g/dl) and past smoker (4.7, 2.7 and 1.93 g/dl). On the other hand, there were no significant differences between Current and past smokers for these parameters. Meanwhile, ALT was significantly increased in current and past smokers as compared to non-smokers, whereas AST was significantly higher in current smoker as compared to non-smokers, while there was no significant difference between current and past smokers as well as between past and non-smokers for this parameter. Previous studies was controversial whether smoking could affect AST and ALT activities. Some investigators claimed ALT was increased by smoking. (Munafo *et al*, 2009), as supported our results, while recent studies argued that smoking did not influence AST or ALT, but GGT. (Sobal *et al*, 2009) Similar results were also reported by Mutwakil Alhibrii *et al*, who studied a number of possible harmful consequences of smoking. Augmented oxidative stress as indicated by low serum uric acid levels and high ALT and AST concentrations were obvious in smokers.(Mutwakil *et al*, 2013)

On the other hand, non-smoker non-diabetics subjects reported a significantly higher mean of total protein, albumin and globulin as compared to all other subjects, where as current smokers diabetics reported a significantly lower mean of these measurements. In contrast, currents smoker's diabetics however recorded a significantly higher mean of ALT and AST as compared to most other interactions, where as non-smoker non-diabetics subjects obtained a significantly lower mean of these parameters.

Total protein, albumin and globulin were significantly higher in past smokers non-diabetics, followed by current smokers non-diabetics. While AST was significantly higher in current and past smokers diabetics than in current and past smokers non-diabetics. Furthermore, the level of ALT was not significantly affected by the diabetes status of smokers, but it was slightly increase in past and current smokers diabetics. These finding were in agreement with Wingerd *et al*, (1977), who found that, current or past coffee consumption and smoking lower serum albumin, globulin, and all other protein fractions.

Diabetics' patients (current smokers, past smokers and non-smokers), they complain of diabetes complications (foot problems (14.3, 13.3 and 6 %), amputation (8.6,6.7 and 0 %) , neuropathy (17.1, 26.7 and 2%) , retinopathy (20, 20 and 8%) ,nephropathy (8.6, 0 and 2 %) and none (31.4,33.3 and 82%), respectively. There were significant differences between the non-smokers diabetics as compared to past

and current smoker's diabetics. This may be due to the effect of cigarettes smoking on glucose deterioration and uncontrol of diabetes. Quitting smoking, generally, had a protective effect against diabetes complications in this study .These agree with similar study in which, current smokers had higher risk of amputation compared with ex-smokers. The prevalence of current smoking among amputees is comparable to the literature, while among diabetic controls is less than that reported .(Idris *et al*, 1998, Suleiman & Ahmed, 2003) The reported prevalence in Sudan is less than that reported in the literature.(Ford *et al*, 1994) In present study, generally diabetes complications were associated with increasing of ALT and AST, whereas associated with decreasing in T.protein, albumin and globulin.

In the present study, correlations existed among smoking duration and number of cigarettes per day for smokers. Reflected (+)ve, medium to weak and significant correlation with AST. But there were (+)ve , weak and insignificant correlation with ALT, while they showed (–)ve, weak and insignificant correlation with total protein ,Albumin and globulin, with an exception that smoking duration showed no significant correlation with AST .There was a study explained that, smoking-related risk of diabetes increases with the number of cigarettes smoked per day.(Will JC *etal*.,2001) Also analyses of data stratified by age and the number of cigarettes smoked per day showed steady increases in CHD mortality rates with increasing duration of smoking for persons younger than age 70 years. Using data from CPS-I, investigators calculated the risk of developing CHD by age and duration of smoking. (Burns *et al*, 1997)

There was (–)ve and significant relationship between smoking duration and no. of cigarettes in one side and smokers total protein , albumin and globulin. Whereas the relationship between these variables and both ALT and AST was (+)ve and insignificant.

The B. value indicates that , for each one unit change in the independent variable (Duration and no. of cigarettes/day), the dependent variables (TB. Albumin, Globulin ,ALT and AST) will change by the value of B. Meanwhile, changing of one year duration of smoking, resulted in change for the above dependent variables by (-0.09) g/dl, (-0.06) g/dl, (-0.03) g/dl, (0.07) IU/L and (0.12) IU/L, respectively. Also in the Physicians' Health Study, a 70% greater risk of diabetes was reported for men who smoked (>20) cigarettes/day than for nonsmokers.(Manson JE etal.,2000) Similar observations were made in women .(Rimm *et al*, 1993, Will *et al*, 2001)

Conclusions:

The present study is the first to describe a detailed effect of cigarettes smoking on liver function of Sudanese subjects. From the results and previous studies, we believed that cigarettes

smoking had different bad effects on smokers and non-smokers. Cigarettes smoking has bad effects on the metabolism of sugars, lipids and proteins. These negative effects may increase the risks of pancreatitis, hepatitis coronary heart diseases, atherosclerosis, different types of cancers and diabetes mellitus type II. These findings place cigarettes smoking on the top causes of mortality and morbidity in the world. People with diabetes already have an increased level of liver enzymes, which will elevate if they smoked. People with diabetes are more likely to have high blood pressure and high levels of fats such as triglycerides. Cigarette smoking may produces insulin resistance and chronic inflammation, which can accelerate diabetes complications, including nephropathy, neuropathy, retinopathy, foot problems and amputation.

Cigarette smoking may produces insulin resistance and chronic inflammation of liver, which can accelerate diabetes complications, including nephropathy, neuropathy, retinopathy, foot problems and amputation. Finally the outcome of these habits, those diabetics' patients can be near to the normal.

RECOMMENDATIONS:

From the results we mentioned the bad effects of cigarettes smoking, which may increase the mortality and morbidity of the participants. To forbid or reduce these risks we recommend that:

1. Smoking cessation is required to save health and community
2. Stopping smoking reduces the risk of hepatitis, cardiovascular disease, lung disease, cancer and stroke.
3. As diabetes also increases the risk for heart disease and stroke, smokers with diabetes are strongly advised to quit.
4. Physical activity and using medication regularly reduce the risks of cigarettes smoking and diabetes
5. Smoking cessation in diabetics decrease diabetes complications
6. For diabetics by following good program such as regular physical activity, using medication in time and good nutrition system, let them look like normal.

References

Abel, G,A, Hays, J. T. Decker, P, A, Kuter,D. J. Rigotti,N. A.(2005). Effect of biochemically confirmed smoking cessation on White blood cell count. Mayo.Clin.Proc80(8):1022-1028.

Burns DM, Smoking and Tobacco Control Monograph No 8. Bethesda (MD): U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute; 1997. pp. 113–304.

Carel, R.S., Eviatar, J.(1985). Factor affecting leukocyte count in healthy adults. Prev. Med. 14(5):607-

Cullen MW, Ebbert JO, Vierkant RA (2009), No interaction of body mass index and smoking on diabetes mellitus risk in elderly women. Preventative Medicine.. 48. 1: 74-78 .

Dallosso HM, James WP. (1984) ,The role of smoking in the regulation of energy balance. Int J Obes;8(4):365–75.

Fagard RH, Nilsson PM (2009), Smoking and diabetes – the double health hazard. Primary Care Diabetes.. 3. 4: 2005-9.

Ford ES, Malarcher AM, Herman WH et al. Diabetes mellitus and cigarette smoking: Findings from the 1989 National Health Interview Survey. *Diabetes Care* 1994; 17: 688-92.

Idris AM, Ibrahim YM, Warnakulasurya KA et al. Toombak use and cigarette smoking in the Sudan: Estimates of prevalence in the Nile State. *Prev Med* 1998; 27: 597-603.

Kalaed Salem Alsalhen, RahabDawoodAbdalsalam. (2014), Effect of cigarette smoking on liver functions: acomparative study conducted among smokers and non smokers male in EL-beida City, Libya, alsalhen and Abdalsalam, international current pharmaceutical Journal, 3(7):291-295.

Klesges RC, Eck LH, Isbell TR, Fulliton W, Hanson CL. (1990), Smoking status: effects on the dietary intake, physical activity, and body fat of adult men. Am J Clin Nutr;51:784 -9.

MathersCD and loncar 2006. Projection of global mortality and burden of disease .plos Med 3(11) 442-443.

Matthew Hilton, "Smoking and Sociability"(2006) in Smoke, p. 133.

Metha, H, Nazzal, K, Sadikot, R. T. (2008). Cigarette smoking and innate immunity.Inflamm. Res. 57(11):497_503.

Moffatt RJ, Owens SG. (1991), Cessation from cigarette smoking: changes in body weight, body composition, resting metabolism, and energy consumption. Metabolism;40(5):465–70.

Molarius A, Seidell JC, Kuulasmaa K, Dobson AJ, Sans S. (1997), Smoking and relative body weight: an international perspective from the WHO MONICA Project. J Epidemiol Community Health;51(3):252–60.

Munafo MR, Tilling K, Ben-Shlomo Y: Smoking status and body mass index:A longitudinal study. Nicotine Tob Res 2009, 11:765–771.

Mutwakil H. Alhibrii, AbdElkarim A. Abdrabo, Mohamed F. Lutfi. Influence of chronic Cigarette Smoking on Serum Biochemical Profile among Sudanese smokers. Asian Journal of Biomedical and Pharmaceutical Sciences, 2013, 3: (18), 17-21.

Nagaya T, Yoshida H, Takahashi H (2008), Heavy smoking raises risk for type 2 diabetes milletus in obese men; but, light smoking reduces the risk in lean men: a follow up study in Japan. Annals of Epidemiology.. 18. 2: 113-8

Nathwani, R,A, Pais, S, Renolds, T, B, Kaplowitz, N, (2005). Serum alanine aminotransferase in skeletal muscles diseases.Hepatology. 41(2):380-382.

Payam O. HusenRezman A Ahmed, (2015),Cigarette smoking risks on blood indices and liver enzymes of male and female smokers in Kurdistan, Iraq, Jordan journal of biological science.

Rimm EB, Manson JE, Stampfer MJ, et al. Cigarette smoking and the risk of diabetes in women. Am J Public Health 1993;83(2):211– 4.

Sander L. Gilman and Xun Zhou(2009), "Introduction" in Smoke: A Global History of Smoking; p. 26.

Sanger Najat Abdul Razaq. (2013), Effect of cigarette smoking on liver function test and some related parameters, Zanco J. Med. Sci. Vol. 17, NO. 3,

Sobal J, Hanson KL, Frongillo EA: Gender, ethnicity, marital status, and body weight in the United States. Obesity 2009, 17:2223–2231.

Suleiman MO, Ahmed ME. Major lower limb amputation in diabetics. *MD thesis; University of Khartoum*, 2003.

Will JC, Galuska DA, Ford ES, Mokdad A, Calle EE. Cigarette smoking and diabetes mellitus: evidence of a positive association from a large prospective cohort study. Int J Epidemiol 2001;30(3):540–6.

Will JC, Galuska DA, Ford ES, Mokdad A, Calle EE. Cigarette smoking and diabetes mellitus: evidence of a positive association from a large prospective cohort study. International Journal of Epidemiology. 2001; 30: 540-6.

Wingerd J, Sponzilli EE: Concentrations of serum protein fractions in white women: effects of age, weight, smoking, tonsillectomy, and other factors. Clin Chem 1977, 23:1310–1317.

Zhong, C. Y. Zhou, Y, M. pinkerton, k. E. (2008). NF-KappaB inhibition is involved in tobacco smoke-induced apoptosis in the lungs of rats. Toxicol. Appl. Pharmacol. 230(2):150-158