Influence of Chronic Cigarette Smoking on Serum Biochemical Profile in Male Human Volunteers

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Electrolytes and minerals are involved in most cellular activities and assume a major role in metabolism. The present study is aimed to understand the influence of electrolyte alterations on serum lipid profile and enzymes in chronic cigarette smokers. Thirty human male volunteers in each group, aged between 27 and 35 taking local diet and smoking for 7–10 years at least 8–12 cigarettes per day were chosen as experimental subjects. All the subjects were using cigarettes without a filter. Controls (age, sex and diet matched) who did not smoke were selected for the study. Blood samples drawn from human volunteers by venipuncture and were used immediately for analysis. There was no significant change observed in serum sodium, chloride levels, where as significant ($p < 0.05$) increase was observed in potassium, iron, calcium and phosphate in chronic cigarette smokers when compared to controls. Further, enhanced activities of the serum enzymes *viz.*, transaminases (Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and with no change in γ-glutamyl transferase (γGT) were observed. The concentration of calcium was positively correlated with serum total cholesterol and low-density lipoprotein (LDL) cholesterol ($r = 0.252$, $r = 0.347$) respectively and negatively ($r = -0.164$) correlated with high-density lipoprotein (HDL) cholesterol, further, potassium was positively correlated with LDH, ALP and ALT ($r = 0.419$, $r = 0.174$, 0.248) respectively in chronic cigarette smokers. Chronic cigarette smoking might have induced alterations in membrane permeability properties of tissues and organs, which might have resulted in changes in signal transduction and electrolyte imbalance.

Key words —— cigarette smoking, mineral, hypoxia, serum enzyme, lipid profile

INTRODUCTION

World wide more than 3 million people currently die each year from smoking, half of them before the age of 70, an enormous human cost, and more than one third have cardiovascular events that often determine permanent disability of affected subjects. There are more than one billion smokers in the world with an increased/decreased/again increased smoking habit.[1)] Smokers are at greater risk for cardiovascular diseases, respiratory disorders, cancer, peptic ulcers and gastroesophageal reflux disease, blindness, bone matrix loss, and hepatotoxicity.[2, 3)] Cigarette smoke contains over 4000 different chemicals, 400 of which are proven carcinogens it also contains various oxidants such as oxygen free radicals and volatile aldehydes which are probably the major causes of damage to biomolecules.[4)] The highly unstable free radicals attack important cellular constituents including DNA, proteins and other opportune targets, particularly cell membranes and the oxidative stress generated is implicated in the pathogenesis.[5, 6)]

Electrolytes and minerals are involved as catalysts in most cellular enzyme catalyzed reactions and assume a major role in metabolism. They have multiple functions such as holding fluids in compartments of the body and maintaining normal acid-base balance, nerve conduction, blood clotting and muscle contractions. Literature survey shows that no sufficient work has been done to study the effect of cigarette smoking on serum electrolyte alterations and its influence on serum lipid profile and enzymes. Electrolyte disturbances may lead to severe and even life-threatening metabolic abnormalities such as coronary heart disease, liver

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Table 1. Characteristics of Controls and Cigarette Smokers

Parameter	Controls	Cigarette Smokers
Age	27–35	27–35
BMI	> 23.5	> 24.2
Number of cigarettes/day	No	8–12
Hypertension	SBP > 130 mm Hg, and/or DBP > 89 mm Hg	SBP > 130 mm Hg, and/or DBP > 89 mm Hg
Blood sugar	80–120 mg/dl (Fasting levels)	80–120 mg/dl (Fasting levels)
Glucose in urine	Absent	Absent
Protein in urine	Absent	Absent
Smoking history	—	7–10
Alcohol consumption	Do not drink	Do not drink
Chronic diseases	Not found	Not found

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

disease, lungs infection, kidney failure, disorders of endocrine system.[7,8] Hence, the present study is aimed to understand the influence of electrolyte alterations on serum lipid profile and enzymes in chronic cigarette smokers.

MATERIALS AND METHODS

Thirty human male volunteers in each group, aged between 27 and 35 residing in Anantapur, India taking local diet and smoking for 7–10 years at least 8–12 cigarettes per day were chosen as experimental subjects. All the subjects were using cigarettes without a filter. Controls (age, sex and diet matched) who did not smoke were selected for the study. All the volunteers were well informed about the experimentation and their written consent was obtained. Institutional ethical committee approved this study. Blood samples from overnight fasted subjects were used for the study. All the volunteers in the present study were free from chronic diseases, illness and use of any tranquillizers, drugs and anaesthetics. Blood samples drawn from human volunteers by venipuncture and were used immediately for analysis. This study was approved by Sri Krishnadevaraya University ethical committee, Anantapur, India.

Estimation of Serum Mineral Content, Enzymes and Lipid Profile —— The serum calcium,[9] phosphorous,[10] iron,[11] sodium,[12] potassium,[13] chloride,[14] and total bilirubin[15] as well as activities of alkaline phosphatase (ALP), lactate dehydrogenase (LDH),[16] γ-glutamyl transferase (γGT),[17] Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)[18] were determined. Serum total cholesterol,[19] triglycerides,[20] high-density lipoprotein (HDL)-cholesterol,[21] low-density lipoprotein (LDL)-cholesterol and very low-density lipoprotein (VLDL)-cholesterol[22] levels were determined.

Statistical Analysis —— Data were subjected to statistical analyses, values are means ± S.D. of 30 subjects in each group. Two-sided paired Student's *t*-test was performed for finding significant difference between the groups. A $p < 0.05$ was considered statistically significant. Correlations between variables were assessed in smokers group with Pearson's correlation coefficients (r).

RESULTS

Table 1 shows the characteristics of controls and cigarette smokers. Table 2 shows minerals (calcium, phosphorous and iron) and electrolytes (Na^+, K^+ and Cl^-) in serum of cigarette smokers and control groups. Cigarette smoking significantly ($p < 0.05$) altered electrolytes and minerals when compared to control group. There was no significant ($p < 0.05$) change observed in serum sodium, chloride levels, where as significant ($p < 0.05$) increase was observed in potassium, iron, calcium and phosphate in chronic cigarette smokers when compared to controls. Enhanced activities of the serum enzymes *viz.*, AST, ALT, ALP, LDH and with no significant ($p < 0.05$) change in γGT were observed in cigarette smokers compare to control group (Table 3).

Levels of cholesterol, triglycerides and lipoprotein patterns in serum of controls and cigarette smokers presented in Table 4. A significant

Table 2. Comparison of Serum Levels of Electrolytes and Minerals between Chronic Cigarette Smokers and Healthy Controls

Parameter	Controls	Cigarette Smokers
Sodium (mM/l)	146.84 ± 3.68	175.98 ± 10.86
Potassium (mM/l)	4.49 ± 0.56	6.42 ± 0.46*
Chloride (mM/l)	101.07 ± 3.27	112.47 ± 1.84
Calcium (mg/dl)	7.98 ± 0.44	9.84 ± 0.51*
Iron (μg/dl)	90.83 ± 5.37	118.26 ± 5.95*
Phosphorus (mg/dl)	3.9 ± 0.4	5.9 ± 0.5*

Values are mean ± S.D. of 30 human volunteers in each group. Student's *t*-test was performed to find out significant difference among groups. $^{*}p < 0.05$ is statistically significant between groups.

Table 3. The Activities of Serum AST, ALT, ALP, LDH, γGT and Total Bilirubin in Chronic Cigarette Smokers and Healthy Controls

Parameter	Controls	Cigarette Smokers
AST (IU/l)	30.64 ± 3.25	44.50 ± 2.87*
ALT (IU/l)	29.96 ± 4.38	54.89 ± 11.02*
ALP (IU/l)	60.52 ± 5.23	84.22 ± 5.43*
LDH (IU/l)	320.16 ± 16.18	429.52 ± 22.47*
γGT (U/l)	5.51 ± 0.60	5.36 ± 0.46
Total Bilirubin (mg/dl)	0.56 ± 0.23	0.17 ± 0.02**

Values are mean ± S.D. of 30 human volunteers in each group. Student's *t*-test was performed to find out significant difference among groups. $^{*}p < 0.05$ is statistically significant. $^{**}p < 0.001$ is statistically significant.

Table 4. Serum Lipid Profile in Chronic Cigarette Smokers and Healthy Controls

Parameter	Controls	Cigarette Smokers
Total cholesterol	161.33 ± 12.1	272.50 ± 10.98*
Triglycerides	119.78 ± 6.1	138.35 ± 5.18*
HDL-Cholesterol	41.35 ± 2.51	23.41 ± 2.86*
LDL-Cholesterol	95.91 ± 4.97	222.6 ± 15.43*
VLDL-Cholesterol	23.84 ± 1.22	27.66 ± 1.62*

Values are mean ± S.D. of 30 human volunteers in each group. Student's *t*-test was performed to find out significant difference among groups. $^{*}p < 0.05$ is statistically significant between groups. All the values are expressed as mg/dl.

($p < 0.05$) increase in serum cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol followed by a significant ($p < 0.05$) decrease in HDL-cholesterol in cigarette smokers is evident from the data.

The relationships between levels of serum calcium and lipid profile as well as potassium and serum enzymes in chronic cigarette smokers were presented in Table 5. The concentration of calcium was positively correlated with serum total cholesterol ($r = 0.252$, $p < 0.012$), LDL-cholesterol ($r = 0.347$, $p < 0.019$) and negatively correlated with HDL cholesterol ($r = -0.164$, $p < 0.001$), further, potassium was positively correlated with LDH ($r = 0.419$, $p < 0.024$), ALP ($r = 0.174$, $p < 0.05$), ALT ($r = 0.248$, $p < 0.026$) in chronic cigarette smokers.

Table 5. The Correlation Analysis between Variables in Chronic Cigarette Smokers

Parameter	Cigarette smokers ($n = 30$)	*P* value
Calcium/Cholesterol	$r = 0.252$	$p < 0.012$
Calcium/Triglycerides	NS	
Calcium/HDL-C	$r = -0.164$	$p < 0.001$
Calcium/LDL-C	$r = 0.347$	$p < 0.019$
Calcium/VLDL-C	NS	
Potassium/LDH	$r = 0.419$	$p < 0.024$
Potassium/AST	NS	
Potassium/γGT	NS	
Potassium/ALP	$r = 0.174$	$p < 0.05$
Potassium/ALT	$r = 0.248$	$p < 0.026$

DISCUSSION

Cigarette smoking is a world-wide major cause of preventable morbidity and mortality.[23–25] Smoking yields chemical substances with cytotoxic potentials.[26] In one puff of a cigarette, the smoker is exposed to more than 10^{15} free radicals in the gas phase alone, with additional exposure in the tar phase equal to more than 10^{17} free radicals per gram.[27,28]

Cigarette smoke consists of many chemicals, including nicotine, tar with its many carcinogens, and gaseous compounds including carbon monoxide (CO).[29,30] CO was shown to accumulated in the human body with repeated smoking.[31] Chronic exposure to low levels of CO results in tissue hypoxia.[32,33] Hypoxia represents a stress that induces cell growth arrest and injury, probably as a result of decreased blood oxygen carrying capacity.[34] Increased carboxyhemoglobin and decreased oxyhemoglobin might have resulted in respiratory acidosis and electrolyte imbalance. Sodium together with potassium assists in the maintenance of the body's electrolyte and water balance.[35,36] In addition, potassium and sodium play an important role in nerve conduction, muscle contraction, and the transport of substances across membranes.[37,38] In the present study no significant change was observed in serum sodium and chloride concentrations in chronic cigarette smokers when compared to controls since people consume enough salt in diet. LDH is an enzyme detectable in cytoplasm in almost every cell of the human body, which becomes extra-

cellular upon cell death. Therefore, its extracellular presence is always related to cell necrosis and tissue breakdown.[39] Increase in the activity of serum LDH and potassium levels may be attributed to the cigarette smoking induced skeletal muscle damage which may leak cellular contents along with potassium and LDH in to the serum.[40] Because the kidneys are the major regulators of external potassium homeostasis, accounting for approximately 80% of potassium transit from the body, renal dysfunction can result in gross abnormalities in serum potassium levels.[7]

Serum iron and red cell hemoglobin concentrations were increased in chronic cigarette smokers when compared to controls. These results are in agreement with previous studies.[32, 41] As tissue hypoxia leads to inadequate oxygenation of blood circulation through lungs results in erythrocytosis and consequent increased production of erythropoietin.[41] Erythropoietin enhances erythropiosis and increases red cell mass above normal level.[42] Increased total red blood cell mass count increases the number of destroyed red blood cells in the normal turnover process which subsequently increases iron over load.[43] This finally leads deposition of excessive iron in the parenchymal cells causing hepatocellular liver damage.[44] Iron over load may amplify the damaging effects of superoxide over production in a very broad spectrum of inflammation both acute and chronic conditions. Furthermore, chronic oxidative stress may modulate iron uptake and storage, leading to a self sustained and ever-increasing spiral of cytotoxic and mutagenic events.[45] Present data shows a significant rise in serum transaminases LDH and ALP activity in chronic cigarette smokers when compared to control groups. Previous studies have reported a significant rise in total serum or tissue specific activity of the above enzymes due to nitrosative stress.[46] The observed low concentration of bilirubin in cigarette smokers when compared to controls may be the activity of hepatic bilirubin Uridine 5 diphosphate (UDP)-glucuronyl transferase (UGT) which can be induced by cigarette smoke.[47]

The components of cigarette smoke are reported to undergo metabolic activation by cytochrome p450 enzymes to form reactive electrophiles, which cause nitrosative stress leading to cytotoxicity mutagenecity and carcinogenicity.[25, 48] Cigarette smoke contains a large number of chemical substances with hepatotoxic potential including nicotine.[26] Cigarette smoke also induces oxidative stress by stimulating NADPH oxidase and decreasing antioxidant defenses, leading to lipid peroxidation.[49] These effects could lead to increased hepatocellular damage and subsequent activation of resident hepatic stellate cells, a major fibrogenic cell-type. In fact, other fibrogenic cell-types such as mesangial cells are stimulated by products from cigarette smoke, such as nicotine to proliferate and produce increased amounts of extracellular matrix proteins.[50] Another potential mechanism by which cigarette smoke leads to liver fibrogenesis may be iron deposition.[51]

Increase in serum calcium levels of chronic cigarette smokers are correlated with increase in plasma lipid profile. Increase in calcium concentration was negatively correlated with serum HDL-cholesterol and positively correlated with LDL-cholesterol. HDL may be involved in the modulation of calcium channels and its decrease might have increased the concentrations of calcium in the plasma of chronic cigarette smokers.[52] Serum calcium may be an independent risk factor for myocardial infarction in middle-aged men followed for 18 years.[53] Cigarette smokers are susceptible to coronary heart diseases.[1] It has been reported that the severity of coronary atherosclerosis is closely related to coronary artery calcification, which itself may correlate with serum calcium and phosphorus concentrations.[54, 55]

In summary, chronic cigarette smoking might have induced alterations in membrane permeability properties of tissues and organs, which might have resulted in changes in signal transduction and electrolyte imbalance. The molecular mechanisms behind these changes and biochemical consequences are needed further in-depth study.

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REFERENCES

1) Aurelio, L. (2005) Biochemical markers of cardiovascular damage from tobacco smoke. *Curr. Pharm. Des.*, **11**, 2190–2208.
2) Witschi, H. (2001) A short history of lung cancer. *Toxicol. Sci.*, **64**, 4–6.
3) Spiro, S. G. and Silvestri, G. A. (2005) One hundred years of lung cancer. *Am. J. Respir. Crit. Care Med.*, **172**, 523–529.

4) Yeh, C.-C., Graham Barr, R., Powell, C. A., Mesia-Vela, S., Wang, Y., Hamade, N. K., Austin, J. H. M. and Santella, R. M. (2008) No effect of cigarette smoking dose on oxidized plasma proteins. *Environ. Res.*, **106**, 219–225.
5) Jose, J. K., Kuttan, R. and Bhattacharaya, R. K. (1998) Effect of Emblica officinalis on hepatocarcinogenesis and carcinogen metabolism. *J. Clin. Biochem. Nutr.*, **25**, 31–39.
6) Srinivasan, M., Sudheer, A. R., Pillai, K. R., Kumar, P. R., Sudhakaran, P. R. and Menon, V. P. (2006) Influence of ferulic acid on gamma-radiation induced DNA damage, lipid peroxidation and antioxidant status in primary culture of isolated rat hepatocytes. *Toxicology*, **228**, 249–258.
7) Jay, N. C., Peter, R. K., Paul, K. W. and Prisant, M. (2000) New Guidelines for Potassium Replacement in Clinical Practice: A Contemporary Review by the National Council on Potassium in Clinical Practice. *Arch. Intern. Med.*, **160**, 2429–2436.
8) John, A. K. (2007) Disorders of acid-base balance. *Crit. Care Med.*, **35**, 2630–2636.
9) Carl, A. B. and Edward, R. A. (1996) Mineral and Bone metabolism. In *Fundamentals of clinical chemistry*, pp. 685–703, W. B. Saunders and company, Philadelphia.
10) Fiske, C. H. and Subbarow, Y. (1925) The colorimetric determination of inorganic phosphorous. *J. Biol. Chem.*, **66**, 375–404.
11) Ramsay, W. N. M. (1958) *Advances in Clinical Chemistry* (Sobotka, H. and Stewart, C. P., Eds., pp. 1–5, Academic Press, New York.
12) Trinder, P. (1951) A rapid method for the determination of sodium in serum. *Analyst*, **76**, 596–599.
13) Jacobs, H. R. D. and Hoffman, W. S. (1931) A new colorimetric method for the estimation of potassium. *J. Biol. Chem.*, **93**, 685–691.
14) Schales, O. and Schales, S. (1941) A simple and accurate method for the determination of chloride in biological fluids. *J. Biol. Chem.*, **140**, 879–884.
15) Laurence, K. M. and Abbott, A. L. (1956) A Micro method for the Estimation of Serum Bilirubin. *J. Clin. Pathol.*, **9**, 270–273.
16) Teitz, R. W. (1976) *Fundamentals of clinical chemistry*, pp. 602–603, W. B. Saunders and company, Philadelphia.
17) Rosalki, S. B. and Tarlow, D. (1974) Optimized Determination of γ-Glutamyl transferase by reaction-Rate Analysis. *Clin. Chem.*, **20**, 1121–1124.
18) Reitman, S. and Frankel, S. (1957) A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Am. J. Clin. Pathol.*, **28**, 8–15.
19) Allian, C. C., Poon, L. S., Chen, C. S. G., Richmond, W. and Fu, P. C. (1974) Enzymatic determination of serum cholesterol. *Clin. Chem.,* **20**, 470–475.
20) Fossati, P. and Principe, L. (1982) Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin. Chem.*, **28**, 2077–2080.
21) Zlatkis, A., Zak, B. and Boyle, A. J. (1953) A new method for the direct determination of serum cholesterol. *J. Lab. Clin. Med.*, **4**, 486–492.
22) Friedwold, W. T., Levy, R. I. and Fredrickson, D. S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin. Chem.*, **18**, 499–502.
23) He, J., Gu, D., Wu, X., Reynolds, K., Duan, X., Yao, C., Wang, J., Chen, C. S., Chen, J., Wildman, R. P., Klag, M. J. and Whelton, P. K. (2005) Major causes of death among men and women in China. *N. Engl. J. Med.*, **353**, 1124–1134.
24) Knoops, K. T., de Groot, L. C., Kromhout, D., Perrin, A. E., Moreiras-Varela, O., Menotti, A. and Van Staveren, W. A. (2004) Mediterranean diet, lifestyle factors, and 10-year mortality in elderly European men and women: the HALE project. *JAMA*, **292**, 1433–1439.
25) Funck-Brentanoa, C., Mathilde, R., Michel, L., Arnould, J.-P., Verstuyft, C., Martine, L., Dominique, C. and Ronan, R. (2006) Effects of type of smoking (pipe, cigars or cigarettes) on biological indices of tobacco exposure and toxicity. *Lung Cancer*, **54**, 11–18.
26) Yuen, S. T., Gogo, A. R., Jr., Luk, I. S., Cho, C. H., Ho, J. C. and Loh, T. T. (1995) The effect of nicotine and its interaction with carbon tetrachloride in the rat liver. *Pharmacol. Toxicol.*, **77**, 225–230.
27) Pryor, W. A. and Stone, K. (1993) Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate and peroxynitrite. *Ann. N. Y. Acad. Sci.*, **686**, 12–27.
28) Richard, J. B., Andrea, K. C. and Webb, A. S. (2007) Physical work-induced oxidative stress is exacerbated in young cigarette smokers. *Nicotine Tob. Res.*, **9**, 205–211.
29) Bokhoven, C. and Niessen, H. J. (1961) Amounts of Oxides of Nitrogen and Carbon Monoxide in Cigarette Smoke, with and without Inhalation. *Nature*, **192**, 458–459.
30) Benowitz, N. L., Hall, S. M., Stewart, S., Wilson, M., Dempsey, D. and Jacob, P., IIIrd. (2007) Nicotine and Carcinogen Exposure with Smoking of Progressively Reduced Nicotine Content Cigarette.

Cancer Epidemiol. Biomarkers Prev., **16**, 2479–2485.
31) Wan-Kuen, J. and Jung-Wook, O. (2003) Evaluation of CO exposure in active smokers While smoking using breath analysis technique. *Chemosphere*, **53**, 207–216.
32) Sagone, A. L., Jr., Lawrence, T. and Balcerzak, S. P. (1973) Effect of Smoking on Tissue Oxygen. *Blood*, **41**, 845–851.
33) Casasola, G. G., Alvarez-Sala, J. L., Marques, J. A., Sanchez-Alarcos, J. M., Tashkin, D. P. and Espinos, D. (2002) Cigarette smoking behavior and respiratory alterations during sleep in a healthy population. *Sleep Breath.*, **6**, 19–24.
34) Zhang, X., Li, J., Sejas, D. P. and Pang, Q. (2005) Hypoxia-reoxygenation induces premature senescence in FA bone marrow hematopoietic cells. *Blood*, **106**, 75–85.
35) Deming, Q. and Gerbode, F. (1953) Observations on sodium balance in patients undergoing mitral valvotomy. *Surg. Forum*, **4**, 18–22.
36) Nguyen, M. K. and Kurtz, I. (2004) Determinants of plasma water sodium concentration as reflected in the Edelman equation: role of osmotic and Gibbs-Donnan equilibrium. *Am. J. Physiol. Renal. Physiol.*, **286**, F828–F837.
37) Hodgkin, A. L. and Huxley, A. F. (1952) The dual effect of membrane potential on sodium conductance in the giant axon of Loligo. *J. Physiol.*, **116**, 497–506.
38) Marsano, L. and McClain, C. J. (1989) Effects of alcohol on electrolytes and minerals. *Alcohol Health Res. World*, **13**, 255–260.
39) De La Pena, V. A., Diz Dios, P. and Tojo Sierra, R. (2007) Relationship between lactate dehydrogenase activity in saliva and oral health status. *Arch. oral biol.*, **52**, 911–915.
40) Montes de Oca, M., Loeb, E., Torrer, S. H., De Sanctis, J., Hernandez, N. and Talamo, C. (2008) Peripheral Muscle Alterations in Non-COPD smokers. *Chest*, **133**, 13–18.
41) El-Zayadi, A. R., Selim, O., Hamdy, H., El-Tawil, A. and Moustafa, H. (2002) Heavy cigarette smoking induces hypoxic polycythemia (erythrocytosis) and hyperuricemia in chronic hepatitis C patients with reversal of clinical symptoms and laboratory parameters with therapeutic phlebotomy. *Am. J. Gastroenterol.*, **97**, 1264–1265.
42) Balcerzek, S. P. and Bromberg, P. A. (1975) Secondary polycythemia. *Semin. Hematol.*, **12**, 339–351.
43) Sagon, A. L., Jr. and Balcerzak, S. P. (1973) Absolute erythrocytosis as a result of smoking. *Clin. Res.*, **21**, 566–571.
44) Bacon, B. R. and Britton, R. S. (1990) The pathology of hepatic iron overload: A free radical-mediated process? *Hepatology*, **11**, 127–137.
45) Emrit, J., Beaumont, C. and Trivin, F. (2001) Iron metabolism, free radicals, and oxidative injury. *Biomed. Pharmacother.*, **55**, 333–539.
46) Chakraborty, A. and Selvaraj, S. (2000) Differential modulation of xenobiotic metabolizing enzymes by vanadium during diethylnitrosamine induced hepatocarcinogenesis in Sprague-dawley rats. *Neoplasma*, **47**, 81–89.
47) Van Hoydonck, P. G., Temme, E. H. and Schouten, E. G. (2001) Serum bilirubin concentration in a Belgian population: the association with smoking status and type of cigarettes. *Int. J. Epidemiol.*, **30**, 1465–1472.
48) Guengerich, F. P., Shimada, T., Yun, C. H., Yamazaki, H., Raney, K. D., Their, R., Coles, B. and Harris, T. M. (1994) Interaction of Ingested Food, Beverage, and Tobacco Components Involving Human Cytochrome P4501A2, 2A6, 2E1, and 3A4 Enzymes. *Environ. Health Perspect.*, **102**, 49–53.
49) Agarwal, R. (2005) Smoking, oxidative stress and inflammation: impact on resting energy expenditure in diabetic nephropathy. *BMC Nephrol.*, **6**, 13–21.
50) Jaimes, E., Tian, R. X. and Raij, L. (2007) Nicotine: The Link Between Cigarette Smoking and the Progression of Renal Injury? *Am. J. Physiol. Heart Circ. Physiol.*, **292**, H76–H82.
51) Bataller, R. (2006) Time to ban smoking in patients with chronic liver diseases. *Hepatology*, **44**, 1394–1396.
52) Stimpel, M., Neyses, L., Locher, R., Knorr, M. and Vetter, W. (1985) High density lipoproteins: modulators of the calcium channel? *J. Hypertens.*, **3**, S49–S51.
53) Lind, L., Jakobsson, S., Lithell, H., Wengle, B. and Ljunghall, S. (1988) Relation of serum calcium concentration to metabolic risk factors for cardiovascular disease. *Br. Med. J.*, **15**, 960–963.
54) Tolstrup, K., Roldan, C. A., Qualls, C. R. and Crawford, M. H. (2002) Aortic valve sclerosis, mitral annular calcium, and aortic root sclerosis as markers of atherosclerosis in men. *Am. J. Cardiol.*, **89**, 1030–1034.
55) Rasouli, M. and Mohseni, A. M. (2006) Serum calcium and phosphorus associate with the occurrence and severity of angiographically documented coronary heart disease, possibly through correlation with atherogenic (apo) lipoproteins. *Clin. Chem. Lab. Med.*, **44**, 43–50.