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Article title

COMBINED EFFECTS OF ORAL CONTRACEPTIVE, ALCOHOL AND CIGARETTE SMOKE ON SERUM LEVELS OF ZINC AND SELENIUM IN WOMEN

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Running Title: Abnormal zinc and selenium levels in women using oral contraceptive.

ABSTRACT
This study was carried out to determine the effect of combined oral contraceptive (OC) on serum zinc, selenium and copper levels in women using COC, consuming alcohol and exposed to cigarette smoke, and to identify if differences in degree of exposure to these agents are capable of modulating effects of COC on antioxidant elements thereby increasing their risks of developing oxidative stress-induced diseases as well as hepatic diseases commonly associated with alcohol consumption. Venous blood samples were taken from the studied population for estimating trace elements and indices of hepatic function. The studied population was divided into smokers\binge drinkers and passive smokers\social drinkers groups. ALT, AST, ALP & copper were not significantly different in exposed group compared with control ($p>0.05$). Both GGT and total bilirubin were significantly increased while zinc and selenium were decreased ($p<0.05$). In addition, among the combined oral contraceptive users, both binge drinkers\smokers and social drinkers\passive smokers groups had a more depleted Zn and Se level compared with non-drinkers\non-smokers group and controls. These results suggest that women on COC consuming alcohol and smoking cigarette may be more susceptible to oxidative stress-induced diseases because of enhanced depletion of antioxidant elements, zinc and selenium.

INTRODUCTION
Oral contraceptives are medications administered by mouth for birth control purposes and are of two main types i.e. the combined oral contraceptive pill and progestogen only pill. Their use was first approved in 1960 in the United States of America (Aiko, 2004; Taylor et al., 2006). According to United Nations (UN) Population Division, 2006, some of the factors which determine its choice as a birth control method include age, education and marital status. The levels of a number of biomolecules have been reported to be influenced by prolonged use of oral contraceptive e.g. Trussell et al. (2007) revealed high doses of estrogen and progestin caused a low level of brain serotonin level by enhancing the level of enzyme that reduces serotonin, low level of serotonin has been linked to depression. Alteration in the levels of coagulation factors has also been reported, thereby affecting coagulation and the risk of deep venous thrombosis (Famodu, 1997; Famodu & Osadebe, 1998). Oral contraceptive use has in some cases been linked to obesity and hypercholesterolemia (Obisesan et al., 2002).

De Groote et al., (2009) revealed that oral contraceptives (OCs) with estrogens as well as progestin may affect oxidative stress (OS) status in human subjects. Results of a number of studies have shown that there were significant differences in the levels of zinc and some other antioxidants in oral contraceptive exposed compared with non-users, even with all these side effects, some beneficial effects have also been identified e.g. its use for the treatment of acne and its ability to reduce risk of ovarian and endometrial cancer (Bast et al., 2007; Arowoju et al, 2009). This study is therefore designed to investigate if exposure to cigarette smoke and alcohol consumption will aggravate the trace element alterations associated with use of COC especially as recent epidemiologic studies have revealed an increase in mortality rate from cardiovascular diseases in people with higher serum copper levels. Either cigarette smoke or alcohol consumption can independently induce free radical ge. eration.

METHODS

Subjects

Thirty females who had been exposed to combined oral contraceptive were randomly selected from Ibadan metropolis, Nigeria and another thirty non-contraceptive users served as the control group while twenty served as combined oral contraceptive users not exposed to either alcohol or cigarette smoke. Apparently, healthy subjects were used for the study. The duration of exposure to combined oral contraceptive was between 5 and 13 years. The mean ages of exposed and control groups are 31.55 and 31.90 years respectively. A well structured questionnaire was administered by a well trained person on all the women individually; subject's age, sex and information about lifestyle, drug history, and existing pathological conditions were obtained from each participant. Women with lifestyle choices, drug history and pathological conditions that may affect the result were excluded. All procedures were in conformity with the ethical standards of our institution on human experimentation and with the Helsinki Declaration of 1975, revised in 1983.

Blood collection and biochemical analyses

Ten milliliter (10ml) of blood was collected from the ante-cubital vein of each member of the combined oral contraceptive and control groups. The blood was collected into non-anticoagulant containing bottles and centrifuged at 3000 r.p.m. for ten minutes to obtain serum. The sera obtained were immediately stored at -20°C until the time required for analysis. Activities of liver enzymes; alanine and aspartate amino transferases (ALT & AST), gama-glutamyl transpeptidase and alkaline phosphatases (ALP); as well as concentrations of total bilirubin and zinc, copper and selenium were determined in the serum of these subjects. ALT & AST activities were estimated using the method of Bergmeyer et al., (1979); ALP by McComb & Bowen (1972) and bilirubin by modified Jendrassik-Grof method (Koch & Doumas, 1982). Hitachi 902 Automated machines (Roche Diagnostic, Germany) was used for the estimation of all other biochemical analytes except Zn, Cu and Se.

For atomic absorption measurements of Zn, Cu and Se, Buck Scientific 205 Atomic absorption Spectrophotometer (Buck Scientific, East Norwalk, Connecticut, USA) was used. Reagents were of high-purity analytical grade (Merck, Darmstadt, BDH, Chemicals Ltd). The water used for the preparation of reagents and working standard was deionized, doubly distilled and re-deionized shortly before use (Millipore Co., Bedford, MA), with specific resistance of > 3 MΩ. Working standard was prepared from the Spectrosol stock standard 1g\L (Buck Scientific). Samples and standards were diluted with a 2mM\L of aqueous solution of Triton X-100 (BDH Chemicals, Ltd). The operating characteristics of atomic absorption spectrometry for the analyzed elements are shown below Table 1.

Statistics

The level of significance between the combined oral contraceptive users and non-users was determined using SPSS package. Student't' test and analysis of variance were used to test the differences between mean values of COC users and controls Analysis of Variance was employed to ascertain level of differences among different sub-groups; namely binge drinkers\smokers, social drinkers\passive smokers and control. Values of $P<0.05$ were considered significant.

RESULTS

The results in **Table 2** are the mean± SEM of ALT, AST, ALP, GGT, zinc, copper, selenium bilirubin total, and bilirubin conjugated as well as the age of the subjects. ALT, AST, ALP; enzymes employed to assess hepatic damage showed non-significant difference between the exposed group and the control group ($p>0.05$). The indicator used to assess exposure to other xenobiotics e.g. alcohol, GGT was significantly higher in COC group compared with control subjects ($p<0.05$). Significant differences were observed for zinc, selenium and total bilirubin ($p<0.05$), zinc and selenium were significantly decreased while total bilirubin was significantly increased. Conjugated bilirubin, on the other hand was not statistically different between the two groups ($p>0.05$). **Table 3** shows the results of inter-group comparison of liver enzymes; ALT, AST and GGT were significantly lower in social drinkers\passive smokers group compared with binge drinkers\smokers group ($p<0.05$). **Table 4** on the other hand shows significant decrease in the levels of zinc, copper and selenium in the smokers group compared with passive smokers.

DISCUSSION

Looking at the results presented in **Table 2**, one can say that the results of most studies carried out on women using COC that showed that COC causes a significant decrease in the level of serum zinc compared with controls (Mc Bean et al., 1977; Crews et al., 1980; Mc Master et al., 1992; Akhter et al., 2005) is in agreement with the result of this study, in which a significant decrease ($p<0.05$) in the serum zinc level was also observed, a pointer that some of the other xenobiotics (alcohol and cigarette) which these subjects were exposed to did not modulate COC effects on zinc metabolism. This modulation was expected because Zhang et al. (2009); Frimpong & Louis (1989) and Clywik et al. (2008) have indicated that alcohol consumption produced higher level of serum Zn while smoking caused no significant change in Zn level. Even without this modulation, the implications of a significant decrease in Zn level in these subjects are diverse; zinc deficiency has been linked to increase in oxidative stress, abnormalities in protein synthesis and in gene expression at both structural and enzymatic levels as well as impaired immune response (Perez-Torres et al., 2009).

Cigarette smoke causes generation of excessive amount of free radical beyond the capability of the en ,enous anti-oxidant system. Apart from this, cigarette smoke also contains other hazardous chemicals such as carbon monoxide, nicotine, tar, benzene, radon and cadmium. The presence of cadmium in the cigarette smoke may be responsible for the significant decrease in the level of selenium in the cigarette smokers group compared with passive smokers and controls. Selenium binds with cadmium in a way suggesting the formation of a 1:1 Cd-Se-protein complex, rendering cadmium less toxic, to facilitate its elimination from the body. Smoking decreases appetite and nutrient absorption in the intestine as well as increases utilization of nutrients such as Zn and Se (Gibson, 1990). Pizent et al. (2003) has also suggested that exposure to cadmium through smoking may contribute to a decrease in serum zinc level.

There was no significant ($p>0.05$) difference in the serum level of copper of combined oral contraceptive users- smoking cigarette and consuming alcohol- compared with the controls. This is contrary to the report of the study of Werbach, (1997); Wynn, (1975) & Berg et al., (1998) who have associated OC use with increased absorption of calcium and copper and with increased blood levels of copper and vitamin A. The modulating effect of these subjects lifestyle choices e.g. alcohol consumption & cigarette smoking which have been reported to cause lowering effect on serum copper level, may be responsible for the non-significant difference, especially as Vir et al. (1981) have reported that smoking caused a significant lowering effect on serum copper values. Moreover, Clywik et al. (2008) have pointed out that excessive alcohol intake causes a number of metabolic changes and disturbs homeostasis of macro- and microelements in the body. Zhang et al., (2009) have also indicated that both alcohol and smoking caused a lower serum copper in alcohol exposed individuals compared with their non- alcoholic counterparts whereas Schuhmacher et al. (1994) from the data obtained from their study concluded that consumption of alcohol significantly reduced the levels of zinc and copper in serum. Copper level though in non-smoking\non-alcohol consuming group was significantly higher than in control subjects.

Furthermore, data emanating from past studies have been inconsistent concerning the effects of oral contraceptive on liver cells. At least, nine case-control studies conducted in developed countries have identified an association between oral contraceptives and a type of liver disease; hepatocellular carcinoma. Some other most recent population-based data from both developed and developing countries, though have failed to confirm such an association (authors unlisted). The non-significant difference observed for both ALT & AST shows that the combined effects of these agents may cause hepatocellular damage.

The significant increase ($p<0.05$) in GGT activity, occurring concurrently with non-significant difference in the activities of ALT & AST suggests that increase in GGT activity might have been resulted from exposure to alcohol. Even with its relatively low sensitivity and specificity, GGT has been recommended as a marker for alcohol use disorders (Balldin et al., 2010).This study is a further confirmation that GGT rather than ALT & AST is a better indicator of alcohol intake. Although the significant increase in the level of GGT might also have been as a result of exposure to cigarette smoke as these subjects were equally exposed to different degrees of cigarette smoke. Wannamethee & Shaper (2010) observed that although cigarette smoking was significantly associated with increased levels of gamma-glutamyl transferase (GGT) and alkaline phosphatase, cigarette smoking does not directly cause liver injury.

Cigarette smoke has also been identified to compound the effects of alcohol in causing liver cell injury in heavy drinkers; smoking affects the liver through inflammatory pathways, thereby aggravating the pathogenic effects of alcohol on the liver, but such effects seem to be absent in this category of subjects as revealed by the results of both hepatotoxic indicators. However, the results of our study is in agreement with that of Adams et al., (Adams et al., 2008) who also identified that ALT was not associated with alcohol consumption in males whereas GGT was significantly associated with alcohol.

Although it is known that the mature adult liver has large reserves of hepatic secretory capability, yet unconjugated hyperbilirubinemia is common in sickle-cell anemia, thalassemia and spherocytosis (Crook, 2006). The significant difference in the levels of unconjugated hyperbilirubinemia in the oral contraceptive group compared with controls might have been as a result of interaction among the different xenobiotics oral contraceptive users were exposed, although the non-significant ($p<0.05$) difference in the activity of AST rules out drug-induced hemolysis as a cause of unconjugated hyperbilirubinemia. Reduction in bilirubin uptake by the hepatocytes seems to be another possible cause of this presentation- unconjugated hyperbilirubinemia.

By sub-dividing the combined oral contraceptive users consuming alcohol and smoking cigarette into binge drinkers\smokers and social drinkers\passive smokers groups, the results in **Tables 3 & 4** once again show the harmful effects of these lifestyle choices (smoking and alcohol consumption), on human subjects. Alanine aminotransferase which was found not be significantly different when the two groups were considered together was significantly increased in binge drinkers/smoker group compared to other groups. Moreover, zinc and selenium were more significantly decreased in binge drinkers/smoker group compared with other groups.

CONCLUSION

One of the most important side effects of oral contraceptive exposure is intravascular thrombosis which has being reported to be mediated by elevation of serum copper level. The non-significant effect observed for copper level in this study shows that this side effect may not be pronounced in the category of subjects recruited for this study. However, care should be exercised in perceiving this as a protective effect since other lifestyle choices of these subjects e.g. smoking and alcohol consumption have been reported to induce oxidative stress leading to depletion in antioxidant levels and therefore increasing the risks for oxidative stress-induced diseases. This increasing risk for oxidative stress-induced diseases was further portrayed by depletion in both zinc and selenium levels. Since cigarette smoke increases the utilization of nutrients and interacted with alcohol to cause a more depleted selenium level than the controls, therefore increase in nutrient requirement values may be considered for this category of subjects.

REFERENCES

Adams LA, Knuiman MW, Divitini ML, Olynyk JK 2008. Body Mass Index Is A Stronger Predictor Of Alanine Aminotransaminase Levels Than Alcohol Consumption J Gastroenterol Hepatol 23(7 Pt 1):1089-93.

Aiko H. (2004-08-20). Japanese Women Shun The Pill. CBS News.

Akhter S, Shamsuzzaman AK, Siddiqui NI, Banerjee M, Hossain MZ 2005. Serum Zinc Status Of Rural Women Taking Combined OC. Mymensingh Med J 14:128-32.

Arowojolu AO, Gallo MF, Lopez LM, Grimes DA, Garner SE 2009. Combined Oral Contraceptive Pills For Treatment of Acne. Cochrane Database Syst. Rev 8:CD004425.

Balldin J, Berggren U, Berglund K, Biennow K, Fahlke C, Zetterberg H 2010. Gamma-Glutamyltransferase In Alcohol Use Disorders: Modification of Decision Limits In Relation To Treatment Goals? Scand J Clin Lab Invest 70(2):71-4.

Bast RC, Brewer M, Zou C, et al 2007. Prevention And Early Detection Of Ovarian Cancer: Mission · Impossible? Recent Results Cancer Res 174: 91100.

Berg G, Kohlmeier L, Brenner H 1998. Effect Of Oral Contraceptive Progestins On Serum Copper Concentration. Eur J Clin Nutr 52:711-5.

Bergmeyer HU, Scheibe P, Wahlefeld AW 1979. Methods For Aspartate And Alanine Amino Transferase. Clin Chem 125: 1487.

Crews MG, Tapper LJ, Ritchey SF 1980. Effects Of Oral Contraceptive Agents On Copper And Zinc Balance In Young Women. Am J Clin Nutr 33: 1940-1945.

Crook AM 2006. Clinical Chemistry and Metabolic Medicine. SeventhEd. London Edward Arnold (Publishers).

Cylwik B, Chrostek L, Szmitkowski M 2008. The Effect Of Alcohol On Iron Metabolism. Pol Merkur Lekarski 24:561-4.

De Groote D, d'Hauterive SP, Pintiaux A, Balteau B, Gerday C, Claesen J, Foidart JM 2009. Effects Of Oral Contraception With Ethinylestradiol And Drospirenone On Oxidative Stress In Women 18-35 Years old. Contraception 80:187-93.

Famodu AA 1997. Serial Changes In Plasma Fibrinogen Concentration And Fibrinolytic Activity In African Women On Oral Contraceptive Pills. Afr. J Reprod Health

1:90-5.

Famodu AA, Osadebe W 1998. Effects of Oral Contraceptives on Coagulation Factors In African Women. Thromb Res 90:27-30.

Frimpong Na, Louis Cj 1989. Copper And Zinc Status In Moderate Alcohol Intake. Adv Exp Med Biol 258: 145-54.

Gibson, R 1990. Principles of Nutritional Assessment. New York, N. Y. Oxford University Press.

Koch Tr, Doumas Bl 1982. Bilirubin: Total And Conjugated, Modified Jendrassik- Grof Method. Am Ass Clin Chem 113.

Mc Bean, Ld, Smith Jc, Halsted Ja 1977. Effects Of Oral Contraceptive Hormones On Zinc Metabolism In The Rat. Pro Soc Biol Med 137: 543.

Mccomb Rb, Bowers Gn, Jr 1972. A Study of Optimum Buffer Conditions For Measuring Alkaline Phosphatase Activity In Human Serum. Clin Chem; 18:97.

Mcmaster D, Mccrum E, Patterson Cc, Et Al 1992. Serum Copper And Zinc In Random Samples of The Population of Northern Ireland. Am J Clin Nutr 56:440-6.

Obisesan Ka, Adenaike Fa, Okunlola Ma, Adenaike Aa 2002. Effects of Oral Contraceptives on Total Serum Proteins, Albumin, Globulins And Cholesterol Levels In Ibadan, Nigeria. West Afr J Med 21(3):197-9.

Pérez-Torres I, Roque P, El Hafidi M, Diaz-Diaz E, Baños G 2009. Association of Renal Damage And Oxidative Stress In A Rat Model Of Metabolic Syndrome. Influence of Gender. Free Radic Res 43:761-71.

Pizent A, Jurasović J, Telisman S 2003. Serum Calcium, Zinc, Copper In Relation To Biomarkers of Lead And Cadmium In Men. J. Trace Elem. Med Biol.; 17:199-205.

Schuhmacher M, Domingo Jl, Corbella J 1994. Zinc And Copper Levels In Serum And Urine: Relationship To Biological, Habitual And Environmental Factors. Sci. Total Environ 148:67-72.

Taylor T, Keyse, Laura Bryant A 2006. Contraception And Sexual Health, 2005/06. London: Office For National Statistics.

Trussell J 2007.Contraceptive Efficacy In Hatcher RA., Et Al. Contraceptive Technology. 19th Rev. Ed. New York: Ardent Media.

Un Population Division. World Contraceptive Use 2005. New York: United Nations. 2006.

Vir Cs, Love AH, Thompson W 1981. Serum And Hair Concentrations of Copper During Pregnancy. Am J Clin Nutr 34:2382-8.

Wannamethee SG, Shaper AG 2010. Cigarette Smoking And Serum Liver Enzymes: The Role of Alcohol And Inflammation. Ann Clin Biochem May 28. [Epub ahead of print]

Werbach MR 1997. Foundations Of Nutritional Medicine. Tarzana, CA: Third Line Press, 210-1 [review].

Wynn V 1975. Vitamins And Oral Contraceptive Use. Lancet; 1:561-4.

Zhang Hq, Li N, Zhang Z, Gao S, Yin Hy, Guo DM, Gao X 2009. Serum Zinc, Copper, And Zinc/Copper In Healthy Residents of Jinan. Biol Trace Elem Res; 131:25-32.

Table 1: The Operating Characteristics of AAS for Zinc, Copper, Selenium, Manganese, Iron, Cobalt, Chromium, Molybdenum and Magnesium

Slit Width	0.7nm(Cu & Zn); 0.2nm (Se)
Wave lengths	Copper (324nm); selenium (196nm), zinc (213nm).
Burner height	Low
Gas mixture	Air – acetylene
Acetylene	12 psi
Air pressure	50 psi
Temperature	2300 °C
Analytical mode	Concentration
Measurement mode	Peak area
Linear range (mg\L)	5.00 (Cu); 2.50 (Zn); 25.00 (Se)
Sensitivity (mg\L)	2 (Cu); 0.5(Zn); 15 (Se)
Detection limit (mg\L)	0.005 (Cu); 0.005 (Zn); 0.5 (Se)
Atomizing air flow	83µl / second
Lamp current	18mA
Scale expansion	3
Noise suppression	2

Table 2: Summarized Mean ±SEM of age and some biochemical parameters of oral contraceptive users and control subjects.

Biochemical parameter (unit)	Oral Contraceptive users	Controls	P value
Alanine aminotransferase (IU\L)	33.35±2.43	32.85±2.55	p>0.05
Aspartate aminotransferase (IU\L)	26.80±1.73	25.75±1.79	p>0.05
Alkaline phosphatase (IU\L)	40.41±1.65	43.02±2.07	P>0.05
Gama-glutamyl transferase (IU\L)	39.20±3.31	22.40±1.28	P<0.05
Copper (µgldl)	109.45±6.41	127.60±7.51	p>0.05
Zinc (µg\dl)	70.95±3.55	94.75±6.07	P<0.05
Selenium (µg\dl)	77.96±3.30	102.44±5.27	P<0.05
Bilirubin-total (µmol\L)	16.50±1.13	11.65±1.06	P<0.05
Bilirubin-conjugated (µmol\L)	7.20±1.05	6.30±0.92	p>0.05
Age (years)	31.55±1.21	31.90±1.36	p>0.05

*significant difference at p<0.05

Table 3: Summarized Mean ±SEM of liver enzymes of different categories of oral contraceptive users and control subjects.

	Binge drinkers\smokers	Social drinkers\passive smokers	Non-drinkers\non-smokers	Controls
ALT (IU\L)*	40.50±2.27	26.20±2.89	30.82±0.53	32.85±2.55
AST (IU\L)	28.60±2.90	25.00±1.87	27.53±0.35	25.75±1.79
ALP (IU\L)	46.14±2.65	35.40±1.94	41.75±1.92	43.02±2.07
GGT (IU\L)*	51.30±3.18	27.10±1.93	23.74±1.66	22.40±1.28

SEM – standard error of mean; ALT-alanine amino transferase; AST-aspartate amino transferase; ALP- alkaline phosphatase; GGT- γ-glutamyl transferase. *- significant difference at $p<0.05$.

Table 4: Summarized Mean ±SEM of age, bilirubin and trace elements of different categories of oral contraceptive users and control subjects.

	Binge drinkers\smokers	Passive smokers\social drinkers	Non-alcohol\non smoking	Controls
Age	31.40±1.56	31.70±1.93	30.56±1.65	31.90±1.36
Copper (μgldl)	102.50±8.18	116.40±9.80	150.10±3.78	127.60±7.51
Zinc (μg\dl)*	67.40±5.65	74.50±4.29	81.04±1.68	94.75±6.07
Selenium (μg\dl)*	72.88±4.73	83.05±3.88	91.20±3.56	102.44±5.27
Bilirubin-total* (μmol\L)	16.90±1.71	16.10±1.56	15.89±0.48	11.65±1.06
Bilirubin-conjugated (μmol\L)	7.50±1.07	6.90±1.88	6.00±0.87	6.30±0.92

SEM-standard error of mean. *- significant difference at $p<0.05$.