RESEARCH COMMUNICATION

Serum γ-Glutamyl Transpeptidase and Alkaline Phosphatase of People in Khon Kaen, the Northeastern Thailand

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Abstract

Serum γ-glytamyl transpeptidase (GGT) and alkaline phosphatase (ALP) of the normal population in Northeastern Thailand were measured. The median serum GGP and ALP levels of females was significantly lower than the values for males, i.e., 21 (range=9-260) versus 32 (range=9-459) and 73 (range= 20-428) versus 83 (range=13-171) U/L, respectively. Serum ALP level tended to increase with age in both male and female populations, whereas serum GGT level did not show any age dependence. Serum GGT and ALP levels of cigarette smokers were significantly higher than those of the non-smokers. Alcohol, coffee or tea drinking also resulted in higher serum GGT value whereas serum ALP values were not changed. Betal nut chewing caused only lower serum GGT but not serum ALP values. The total population in Ban Fang district seemed to have lower serum GGT than those in Chonnabot district. The results from our study give the base line data of serum GGT and ALP in a Thai population. We also confirm the association of serum GGT with cigarette smoking, alcohol and coffee/tea drinking which are risk factors of cancer.

Key Words : alkaline phosphatase - γ-glutamyl transpeptidase - serum - Thailand

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Introduction

Serum biochemical parameters are associated with several diseases, including cancers. Gamma Glutamyl transpeptidase (GGT, EC 2.3.2.2), a major enzyme of glutathione (GSH) homeostasis, is located on the external surface of epithelial cells exhibiting large secretory or detoxification activities, and it catalyzes the transfer of a γ-glutamyl group from a γ- glutamyl peptide to an acceptor peptide or an L-amino acid (Tate and Meister, 1981; Hanigan,1998). GGT is an important biological marker for hepatobiliary diseases (Stark, Pagano, Zeiger, 1991; Matsuo et al 1999; Myers et al, 2003) and its expression is enhanced in sereral carcinomas (Hanigan et al, 1994; Nishimura et al, 1998). GGT also mediates utilization of extracelluler glutathione contributing to protection against oxidative injury (Forman, Skelton, 1990; Shi et al, 1993). Alkaline phosphatase (ALP, EC 3.1.3.1) is a group of heterogeneous isoenzymes that catalyze the hydrolysis of monophosphate esters at alkaline pH with a wide range of substrate specificities (Chang et al 1994; Calhau et al, 2000). Serum alkaline phosphatase is used in the diagnosis of hepatic and bone lesions, and it is also related to several cancers' growth rate including hepatocellular carcinoma (Stillwagon et al 1991; Chevret et al 1999).

These two enzmes are most widely regarded as the best indicators of chronic alcohol consumption (Nishimura and Teschke, 1982). Because it is reported that the high incidence of liver cancer in the Northeastern poputation of Thailand might have a relation to life style, and behavior of dietary intake as well as other risk factors (Vatanasapt et al 1990a; 1990b; Sriamporn et al, 1993), we have focused on measuring the serum levels of GGT and ALP, to provide baseline data for people in the Northeast of Thailand.

Materials and Methods

1. Sample Collection

The blood samples used in this study were obtained from people in Chonnabot and Ban Fang Districts of Khon Kaen

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Province who had no disease history and participated in a mobile cancer screening programme during 1990. Those found with any abnormality from a physical investigation, such as oral cavity mass, breast mass, thyroid gland enlargement or from ultrasonography and Pap smear were excluded from this study.

Information of tobacco smoking, betel nut chewing and alcohol drinking was obtained from questionnaires.

Overnight fasting venous blood was drawn, transferred to a microtube and kept in an ice-box. When the samples arrived at the laboratory in the University, the serum was immediately separated, and stored at –80°C until used for enzymatic analysis, which was within a week of the sample collection.

2. Chemicals

γ- glutamyl- p-nitroanilide (GPNA), glycylglycine, TRIS, p-nitrophenyl phosphate, and glycine were obtained from the local distributor of Sigma Chemical Company.

3. Enzymatic Analysis

Serum GGT and ALP were measured by autoanalyzer (Ciba Corning, Express 550). GGT activity was assayed according to the method described by Silber et al (1986), using γ- glutamyl-p-nitroanilide as a substrate. ALP activity was assayed by the modification method of Bowers and Mc.Comb (1966), using p-nitrophenyl phosphate as a substrate. The unit of GGT or ALP activity was espressed in terms of 1 mmol of p-nitroaniline formed, or p-nitrophenyl phosphate disappearing per minute per liter (U/L) of sample, respectively.

4. Statistical Analysis

All the group data were statistically evaluated and significant differences by various factors were determined using non-parametric statistical methods (Hollander and Wolfe, 1973). The results are expressed as median (range) of each group. The level of statistical significance was set at $p<0.05$.

Results

As shown in Table 1, the serum γ-glutamyl transpeptidase (GGT) levels of 347 blood samples collected from people in Ban Fang and Chonnabot districts of Khon Kaen, the northeast of Thailand, were gender different. The median GGT level of the total population was 26 (9-459)U/L. The female serum GGT was significantly lower than those of male, the median levels were 21 (9-260) and 32 (9-459) U/L, respectively ($p<0.05$).

The results from the determination of serum alkaline phosphatase (ALP) of 337 blood samples are shown in Table 2. It was also revealed that the median female serum ALP was significantly lower than the values of male, which were 73 (20-428) and 83 (13-171) U/L, respectively ($p<0.05$). The median value of total population was 79 (13-428) U/L. In contrast to serum GGP, the serum ALP in both sexes was clearly age different. When the age increased, the values in males increased as observing that the median value of ≤ 35 years group is significantly lower than the value of 36-45 and 56$^+$ years groups, which were 68.5 (23-89), 82.5 (37-165) and 88.5 (20-165) U/L, respectively ($p<0.05$). Similarly, the age difference of serum ALP was revealed in females. The median value of the ≤ 35 years group was significant lower than those values of the 56$^+$ years group, i.e., 64 (48-90), versus 91 (20-428) U/L.

The analysis of a sample of the population in relation to their various behaviors is demonstrated in Table 3. There is increased serum GGT in those who smoke or drink alcohol, tea or coffee. The median values of serum GGT of the total population were higher in cigarette smokers than in non-smokers, which were 32 (9-174)and 22 (9-260) U/L, respectively ($p< 0.05$). The median serum ALP of total population was also higher in the smoking group than in non-smokers, which were 83 (23-171) and 74.5 (13-217) U/L, respectively ($p< 0.05$). It is important to note that 42% of the total population smoked cigarettes, and almost all of them (96%) were males.

For alcohol drinking behavior, which was found to be 50% of total population, 75% of them were males. The median serum GGT of alcohol drinkers was significantly higher than that of non-drinkers, which were 28.5 (9-260) versus 24 (9-212) U/L, respectively. However, the difference was not detected for serum alkaline phosphatase.

For coffee or tea drinking behavior which was 15% of total population, the median serum GGP of drinkers was higher than for the non-drinkers, i.e., 33 (10-111) versus 25 (9-260) U/L ($p< 0.05$). In contrast, we did not find any

Table 1. Median Serum γ-glutamyl Transpeptidase of People in Khon Kaen in Various Age Ranges

Age	Male		Female		Total	
	No	Median (range)	No	Median (range)	No	Median (range)
≤ 35	11	34.0 (16-108)	18	24.0 (15-80)	29	26.0 (15-108)
36-45	75	33.0 (11-162)	72	20.0 (10-260)	147	25.0 (10-260)
46-55	45	31.0 (9-459)	54	23.0 (10-126)	99	27.0 (9-459)
56+	32	31.5 (18-174)	40	22.5 (9-96)	72	26.5 (9-174)
Total	163	32.0 (9-459)	184	21.0 (9-260)[a]	347	26.0 (9-459)

Values expressed in terms of U/L [a] significant difference from males ($p<0.05$)

Table 2. Median Serum Alkaline Phosphatase of People in Khon Kaen, the Northeastern Thailand at Various Age Ranges

Age	Male		Female		Total	
	No	Median (range)	No	Median (range)	No	Median (range)
≤ 35	10	68.5 (23-89)	17	64.0 (48-90)	27	68.0 (23-90)
36-45	72	82.5 (37-165)[a]	71	69.0 (37-131)	143	76.0 (37-165)
46-55	44	83.5 (13-171)	54	80.5 (30-166)[a]	98	83.0 (13-171)[a]
56+	30	88.5 (20-165)[a]	39	91.0 (20- 428)[a,b]	69	89.0 (20-428)[a,b]
Total	156	83.0 (13-171)[c]	181	73.0 (20-428)	337	79.0 (13-428)

Values expressed in terms of U/L [a,b,c] significant difference when compared to ≤35 and 36-45, and female values, respectively ($p<0.05$)

differences in the serum ALP levels between coffee or tea drinking and the non-drinking group. For betel nut chewing which was 26% of the total population where most of them are female (95%), we demonstrated a significant difference of serum GGT when compared to the non-chewing group. The serum GGT of betel nut chewing was lower than the value of non-chewing which were 22.5 (9-260) and 27 (9-212) U/L, respectively ($p<0.05$). Serum ALP of betel nut chewers was not significantly different for non-chewers.

When the serum GGT and ALP of people in 2 districts was compared, the values of Chonnabot were slightly higher than those of Ban Fang with age and sex adjustment.

Discussion

The results obtained in this study indicate the gender differences in serum liver enzymes of people of Northeastern Thailand. Serum GGT and ALP levels of males were higher than those of females. Age differences were observed only for serum ALP but not for serum GGT in both males and females, reflecting the age-related nature of alkaline phosphatase in normal population. Since human alkaline phosphatases are isozymes and originate from four major organs including hepatic, intestine, bone and placenta (Millan and Fishman, 1995); and the earlier reporting of elevated placental ALP may enable early diagnosis of ovarian cancer in females (Ben-Arie et al,1999), the further determination of specific type of serum ALP may suggest the involved organ sources in the older ages of both sexes. Although it was surprising to find increased serum GGT in cigarette smokers, alcohol and coffee or tea drinkers, whereas increased serum ALP only in cigarette smokers, we confimed the previous findings that chronic alcohol consumption significantly enhances the serum GGT and ALP (Nishimura and Teschke, 1982). Our findings were not similar to the previous report of Tanaka et al (1998) which demonstrated the inverse relation between coffee or green tea drinking with serum GGT of males in Japan. This may reflect the different types of coffee and tea consumed in Thailand to those did in Japen.We demonstrated that serum GGT is a more sensitive biochemical marker than serum ALP in association with alcohol drinking. This may be explained

Table 3. Median Serum γ-glutamyl Transpeptidase and Alkaline Phosphatase of People in Khon Kaen with Various Behaviors

Variables	GGT		ALP	
	Number	Median (range)	Number	Median (range)
1. Cigarette				
Smoking	145	32 (9-174)[a]	139	83 (23-171)[a]
Non-smoking	200	22 (9-260)	196	74.5 (13-217)
2. Alcohol				
Drinking	180	28.5 (9-260)[a]	174	80.5 (13-171)
Non-drinking	164	24 (9-212)	160	76.0 (20-217)
3. Coffee/Tea				
Drinking	63	33 (10-111)[a]	62	81.5 (13-171)
Non-drinking	281	25 (9-260)	273	78.0 (20-217)
4. Betal nut				
Chewing	86	22.5 (9-260)[a]	82	81.0(20-217)
Non-chewing	259	27 (9-212)	253	78.0(13-171)
5. District				
Ban Fang	151	23 (9-90)[a,b]	151	77.0(30-176)[b]
Chonnabot	195	28 (10-260)	185	79.0(13-217)

[a] significant difference from non-smoking, non alcohol and non coffee-drinking, non betal nut-chewing ($p<0.05$)
[b] significant difference after adjustment for age and gender ($p<0.05$)

by alcohol and its metabolites increase serum GGT by its hepatotoxic or by reducing the antioxidant levels (Crawford, Balakenhoan,1991), which is the promotor for hepatocirrhosis. Our results supported the notion that elevation of serum GGT precedes that of ALP in the early stages of primary biliary cirrhosis (Matsuo et al, 1999). Therefore serum GGT is very useful for early detection of hepatic related disease. We found higher serum GGT and ALP of people in Chonnabot district than those in Ban Fang district. These findings were inversely associated with our study on serum antioxidant vitamins that were found to have higher levels in Ban Fang district than in Chonnabot district (Sripanidkulchai et al, 2003). These results confirmed the report of cancer incidence from the Khon Kaen population-based cancer registry that showed the incidence of cancer of Channabot district was higher than that in Ban Fang district. Moreover the cancer incidence in males is higher than in females in both districts (Vatanasapt el at, 1998). It is also important to note that the majority of cigarette smoking, alcohol and coffee/ tea drinking individuals were males, which may reflect the behavioral factors that are very important for cancer incidence in the male population.

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