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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. *Antimicrob. Agents Chemother.* 51: 1281-1286.

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## Full Length Research Paper

# Prevalence of antibody titre in healthy individual and enteric fever patients in Owerri, Nigeria

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The aim of this study was to establish the prevalence of enteric fever amongst individuals in Owerri municipal area of Imo state. The study shows that 61 (24.4%) patients had typhoid fever based on clinical examination and serological test results (Widal test). It was observed that prevalence of enteric fever was high among the patients of age group 26 to 35 years (44.3%). Similarly, prevalence of typhoid fever was higher in females with 38 (62.7%) than males 23 (37.7%). It was concluded that prevalence of typhoid fever was high among the youths who consume unsafe drinking water and food from outside source. However, the survey revealed that not all medical cases of typhoid fever reported were correct because most private medical clinics do not carry out tube agglutination test on their patients due to lack of medical facilities and poverty level in the country.

**Key words:** Antibody titre, typhoid, enteric fever, drinking water.

## INTRODUCTION

Enteric fever is an acute systemic infection of humans caused by *Salmonella enterica* serovar Typhi (S. Typhi) (Olsen et al., 2004) and *Salmonella enterica* serotype Paratyphi A, B and C (S. Paratyphi A, B and C) also known as non typhoid salmonella (NTS) causing similar type of condition with less severity (Bhan et al., 2005). The fever is a global infectious disease with prevalence in Africa, South America and greatest risk in the Indian subcontinent (Bhutta and Hendricks, 1996; Parry, 2005). The annual incidence of enteric fever is estimated to be about 17 million cases worldwide (World Health Organization (WHO), 2008). In Africa, it has an estimated crude incidence of 362 cases per 100,000 persons per year (Buckle et al., 2012). In most endemic areas,

approximately 90% of enteric fever is typhoid and caused about 216,500 deaths among children and young adults worldwide (Kothari et al., 2008; Yang, 2008).

Typhoid and paratyphoid fever causes serious public health problem in developing countries due to their poor standard of hygiene and unavailability of potable water (Crump et al., 2004; Nicholas et al., 2010). It is mostly encountered in tropical and sub-tropical countries including Nigeria where it constitutes significant sources of morbidities and mortalities (Ibekwe et al., 2008).

These bacteria can survive between days to months in seawater and contaminated eggs, respectively (Elsarnagawy, 1978; Wait and Sobsey, 2001; Kothari et al., 2008). The infectious dose of enteric organisms in

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human can vary between  $10^3$  to  $10^6$  organisms when taken orally via ingestions of food or water contaminated with faeces (Kothari et al., 2008). Also, it can be transmitted through recent contact with a typhoid patient or carrier, eating ice cream flavoured iced drinks or food from street vendors and raw fruit and vegetables grown in field fertilized with sewage (Bhan et al., 2005). Infected individuals serve as reservoir for the bacteria and source of infection to others in the transmission mode of enteric fever. Within one week of infection, the bacteria enter into the blood and shed through stool. At the second week, it appears in both stool and urine.

In Nigeria, the facilities for isolation and identification of these organisms in most private hospitals and medical laboratories are rarely available (Onunkwo et al., 2001). Many hospitals in rural areas lack facilities for blood culture, therefore up to 90% of patients with fever are treated as outpatients. This situation has made it difficult to obtain reliable data from which to estimate the extent of the disease in country. The disease is characterized by malaise, fever, abdominal discomfort, transient rash, splenomegaly, hepatomegaly, bradycardia and leucopenia; the most prominent major complications are intestinal hemorrhage and perforation (Hook and Gurrant, 1987). The standard diagnosis of enteric fever is by isolation of the organism from culture of blood, bone marrow aspirate, stool, bile or urine.

Typical reactions of *S. enterica* serovar Typhi include a characteristic biochemical pattern in triple sugar iron medium which produces acid but without gas, an alkaline slant and a reasonable amount of  $H_2S$  production tests. Identification is confirmed by serological demonstration of the lipopolysaccharide "O" antigen of group D, protein flagella antigen Hd and Vi polysaccharide of capsular antigen (Parry, 2005). This study reports on frequency distribution of healthy individuals against positive typhoid fever cases and distribution frequency of *S. enterica* serovar Typhi / Paratyphi antigens in positive patients. It also establishes the prevalence of enteric fever amongst gender and age distributions.

## MATERIALS AND METHODS

### Samples collection

The blood samples were collected from Private medical centre laboratories, private clinics in Owerri and Federal University of Technology Owerri. Most of these samples collected from the Private clinics and Medical laboratories were those used to investigate suspected cases of "typhoid fever". The samples collected from the university community included those of healthy individuals. The blood samples were collected with sterile bijoux bottles, serum later extracted and stored in refrigerator prior to screening. Each serum sample was accompanied by a predesigned questionnaire with the following information – age (5 to 30 years), sex and date of collection. All samples were collected between January and April, 2013. A total of 250 blood samples was collected and investigated. The number of supposed typhoid fever patients collected from various clinics and private medical laboratories was

190 while 60 individuals from the University community donated their blood samples, making up the total of 250 samples used for this research.

### Widal agglutination test

Widal agglutination test was performed using widal agglutination kit (Biotech lab, united states) containing both somatic "O" and flagella "H" antigens of *S. Typhi* and *S. Paratyphi* A, B and C. A negative saline control was introduced in each batch of test. The widal test was carried out in three phases namely rapid slide agglutination, tube dilution and confirmatory tube agglutination tests.

### Rapid slide agglutination

All the sera samples were initially analysed for *S. Typhi* antibodies on a clean greaseless tile. Drop of widal antigen suspension antigens were placed unto undiluted sera samples, mixed by gently rotating the tiles for 1 to 2 min and observed for agglutination. The sample that showed granules (agglutination) with any of the antigen suspension on the tile was considered positive and set aside for tube agglutination test.

### Tube dilution agglutination test

Dilution of the serum (1:10) was made with the test antigen suspension in test tubes. The presence of positive tube agglutination test necessitated confirmatory test to the determine antibody titre value. The resultant positive sera samples were further tested at 1/20, 1/40, 1/80, 1/160 and 1/320 in 0.8% normal saline. The tubes were incubated in a water bath at 45 to 50°C for 18 h when somatic "O" antigen was used while the tubes were incubated for one hour at the same temperature when flagella "H" antigen was used. The tube in which there were granules formations were regarded as positive tube agglutination test, while the ones that the suspension remained cloudy gave negative results. A positive widal test was considered as one that gave a reaction titre from 1/80 and above in a single test.

## RESULTS AND DISCUSSION:

Table 1 shows 95 sera samples from private medical laboratory, 83 samples from private clinics and 15 samples from Federal University of Technology Owerri (FUTO) community, making a total of 201 sera samples screened as positive on rapid slide agglutination test, while 61 samples showed positive agglutination test with titre  $> 1/180$  and this represents 24.4% of the entire individuals tested. This contradicts the report of Esohe et al. (2012) who reported 73.9% with signs compatible with typhoid fever among 234 patients tested in Ikare Akoka, Ondo state of Nigeria.

Table 2 shows the age distribution pattern of typhoid fever. The age of 26 to 35 years (27 cases) showed the highest positive result while the least positive result was found in the age group of 5 to 15 years (4 cases) amongst total of 61 samples that showed positive tube agglutination tests. This concurs with findings of Esohe et al. (2012) who reported 66 patients out of 173 patients

**Table 1.** Frequency distribution of healthy individuals against positive typhoid fever cases.

| Sampling location          | No. Of Healthy individual | Frequency of Supposed Typhoid fever sera samples | No. Positive Screening tests (%) | No. of confirmed positive tube agglutination (%) |
|----------------------------|---------------------------|--|----------------------------------|--|
| FMC                        |                           | 8  | 8 (3.2)                          | 7 (2.8)  |
| Private medical laboratory | -                         | 98   | 95 (38.0)                        | 26 (10.4)  |
| Private clinic             | -                         | 84   | 83 (33.2)                        | 30 (12.0)  |
| FUTO                       | 60                        | -  | 15 (6.0)                         | 5 (2.0)  |
| Total                      | 60                        | 190  | 201                              | 61 (24.4)  |

Total no of patients tested = 250, FMC = Federal medical centre.

**Table 2.** Age distribution pattern of typhoid fever cases.

| Age (years) | No. tested | Frequency of Positive tube agglutination tests | % Typhoid fever patients |
|-------------|------------|--|--------------------------|
| 5-15        | 25         | 4  | 6.6                      |
| 16-25       | 68         | 12   | 19.7                     |
| 26-35       | 97         | 27   | 44.3                     |
| 36-45       | 32         | 13   | 21.3                     |
| 46-60       | 28         | 5  | 8.2                      |
| Total       | 250        | 61   | 100                      |

**Table 3.** Gender distribution pattern of typhoid fever cases.

| Gender | No tested | Frequency of Positive tube agglutination tests | % Typhoid fever patients |
|--------|-----------|--|--------------------------|
| Male   | 105       | 23   | 37.7                     |
| Female | 145       | 38   | 62.3                     |

who tested typhoid fever positive to be within the range of 26 to 35 years while reports of Nicholas et al. (2010) and Parry (2005) showed that prevalence of typhoid fever in endemic areas is considered high in school-aged children and young adults. Older adults are presumably relatively resistant due to frequent boosting of immunity (Parry, 2005).

Table 3 defines the prevalence of *S. Typhi* infection amongst gender of the patients' symptoms. The highest typhoid positive result is shown in 38 female patients while 23 males representing 62.3 and 37.7%, respectively. This may be due to the fact that females are more vulnerable to such disease due to poor health conditions and environmental factors associated to these women. This is mostly found amongst market women who are not aware of the mode of transmission of this disease due to poor level of education. The result is contrary to earlier report of Ibekwe et al. (2008) who reported typhoid titres above 80 to occur more in male 3.7% against 3.6% of females.

Table 4 represents the distribution frequency of *S. enterica* serovar Typhi/Paratyphi antigens in positive

patients. A total of 151 cases of typhoid fever somatic 'O' and 'H' agglutinin titer levels were assessed; 31 (20.53%) had an 'O' agglutinin titer level above 1:160 while 10 patients (6.62%) had an 'H' agglutinin titer level above. However, a significant number of typhoid cases was obtained by 'O' and 'H' titers together 110 (72.85%). Somatic O antigens serologically were more common causes of typhoid fever in Owerri municipal council, with *S. Typhi* being the most prevalent with 34.4% patients being sero-positive for antibodies, *S. Paratyphi* B-H antigens closely followed with 16 positive typhoid fever patients, representing 26.2% prevalence in Owerri. The least was observed with *S. Paratyphi* C-H antigens showing 4.9% prevalence. Earlier study by Esohe et al. (2012) and Ibekwe et al. (2008) proved somatic "O" antigens to be common cause of typhoid fever in different parts of Nigeria. Ibekwe et al. (2008) also reported *S. Typhi* 'O' to have shown positive titre of 39% in male and 10.7% in female when tests in apparently normal individuals were conducted.

Variation in geographic location is an important tool in the epidemiology of enteric fever and could help in

**Table 4.** Distribution frequency of *S. enterica* serovar Typhi/Paratyphi antigens in positive patients.

| Antigen      | Serotypes                | No. of positive tests | % positive test |
|--------------|--------------------------|-----------------------|-----------------|
| Somatic "O"  | Salmonella Typhi O       | 21                    | 34.4            |
|              | Salmonella Paratyphi A-O | 15                    | 24.6            |
|              | Salmonella Paratyphi B-O | 6                     | 9.8             |
|              | Salmonella Paratyphi C-O | 8                     | 13.1            |
| Flagella "H" | Salmonella Typhi H       | 10                    | 16.4            |
|              | Salmonella Paratyphi A-H | 11                    | 18.0            |
|              | Salmonella Paratyphi B-H | 16                    | 26.2            |
|              | Salmonella Paratyphi C-H | 3                     | 4.9             |

Total No. of positive enteric fever patients based on tube agglutination test = 61.

**Table 5.** Survey of typhoid fever cases in Owerri.

| Months (2013) | FMC typhoid fever cases | Private clinic typhoid fever cases | Private medical laboratories typhoid fever cases |
|---------------|-------------------------|------------------------------------|--|
| January       | 1                       | 37                                 | 45   |
| February      | 1                       | 26                                 | 12   |
| March         | 4                       | 8                                  | 24   |
| April         | 3                       | 13                                 | 17   |
| Total         | 8                       | 84                                 | 98   |

diagnosis. This means that some serotypes are known to be more prevalent in certain areas than in others due to exposure to modern facilities.

Table 5 shows the survey carried out on typhoid fever cases from hospitals located in Owerri Municipal council Imo state, Nigeria from January to April, 2013. Private medical laboratories recorded the highest number of patients diagnosed of the disease. The report agrees with previous reports of Erdem et al. (2004) who reported sampling of typhoid fever patient from outpatient clinics as 63.2 and 36.8% hospitalized patients. Raffatellu et al. (2008) also reported increase in the frequency of enteric fever with serovar Paratyphi A showing a wider spread in some parts of the world while Asia has the highest incidence rates of typhoid fever. This may be attributed to self medication amongst the inhabitant of the city; similarly many private clinics operate with in-house medical laboratories. It was also observed that the Federal Medical Centre with qualified medical personnel had 8 patients diagnosed of the typhoid fever within the period under review. This frequency may be attributed to availability of modern scientific equipment in Federal Medical Centre unlike Private Medical Laboratories.

## Conclusion

The frequency of survey made during this research stimulated the quest to prove whether all diagnosed of

typhoid fever were actually suffering from the disease or related symptom that may be associated with latent clinical infection. From the total sample of 190 "Supposed typhoid fever patient" and 60 "healthy individual" tested, it was observed that only 61 representing 24.4% individuals were positive to Widal tube agglutination test. From this result, it can be deduced that most test carried out are rapid slide agglutination test which can give "false positive result" based on prolonged illness or non typhoid Salmonellosis. Also, most cases of malaria had often diagnosed and treated as typhoid fever due to inexperienced medical laboratory personnel and non availability of modern scientific equipment. Therefore, government should also embark on enlightenment campaigns for health workers and training sessions for most private medical laboratory owners. Also, it is important to create awareness on the importance of personal health hygiene and the risks associated with non compliance.

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## Full Length Research Paper

# Epidemiology of schistosomiasis in school aged children in some riverine areas of Sokoto, Nigeria

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**Prevalence of Schistosomiasis in primary school pupils in riverine areas of Sokoto, where most of the population is dependent on river and well water for their everyday activities, was surveyed using stool and urine samples. The stool samples were analysed using kato-katz thick faecal smear technique while the urine samples were processed by filtration technique. The overall prevalence of urinary schistosomiasis (*Schistosoma haematobium* Leiper) was 60.8% (228 positive cases in 375 samples), and for intestinal schistosomiasis (*Schistosoma mansoni* Leiper) was 2.92% (11 positive in 375 samples). Prevalence of disease vary among age and sex of pupils. Pupils of age group 9 to 12 years are highly prevalent (71.42% for urinary and 4.2% for intestinal schistosomiasis). Prevalence of disease was high among males 79.57% (187 positive in 235 samples) urinary and 3.80% (7 positive out of 189 samples) intestinal schistosomiasis in comparison to females with a prevalence rate of 29.28% (41 positive in 140 samples) and 2.15% (4 positive out of 186 samples), respectively. Prevalence in the studied area is therefore very high and of family status, sex and age dependent.**

**Key words:** Helminth parasites, schistosomiasis, *Schistosoma mansoni*, *Schistosoma haematobium*, snails.

## INTRODUCTION

Schistosomiasis (Bilharzia) is a water born parasitic disease caused by *Schistosoma*, the digenic trematode found in the blood vessels of man and livestock. Schistosomiasis is a chronic, debilitating parasitic disease infecting more than 200 million people and is second only to malaria in terms of public health importance. About 95% of African population is infected with the disease (Bello et al., 2003). In Nigeria, the incidence of schistosomiasis is so common in some communities that young men passed the bloody urine at some stage of

the disease (Eni et al., 2008). There are several complications of chronic urinary schistosomiasis such as bladder cancer (Eni et al., 2008), which is the major cause of morbidity and mortality in endemic areas. Studies also suggest that HIV/AIDS is highly prevalent in the areas of parasitic worm infections, such as schistosomiasis (Bentwich et al., 1995).

In Nigeria, about five species (SPP) of the genus *Schistosoma* are pathogenic to man. These species (SPP) include *Schistosoma haematobium*, *Schistosoma*

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*mansoni*, *Schistosoma japonicum* (Miyari and Suzuki), *Schistosoma intercalatum* and *Schistosoma Mekongi* (Theodore Bilharz) (Uko et al., 1993; Agi and Okafor, 2008). Among which three species *S. haematobium*, *S. mansoni* and *S. japonicum* account for more than 95% of all human cases of schistosomiasis found in the world (Mutapi et al., 2003). The disease caused by *S. haematobium* is characterized by bloody urine, lesion of bladder, kidney failure and bladder cancer in children (Butterworth, 1997) and is the major cause of female genital schistosomiasis (FGS), which is a risk factor for transmission of sexually transmitted diseases and HIV (TDR, 1996); while the *S. mansoni* infection is characterized by bleeding from gastro – oesophageal region, splenohepatomegaly, growth retardation, delayed sexual maturity and chronic dermatitis (World Health Organization (WHO), 1998).

Though the disease kills few people, its clinical effects, prevalence and association with other diseases and expansion of agriculture and water development projects, movement of population and increase in population density and some social habits like passing urine and faeces near water bodies makes it a problem of great health importance (WHO, 2010). In the present study attention has been focused on the epidemiological survey of the disease in local vulnerable population with the broader objective of control programme for schistosomiasis in the affected areas.

## MATERIALS AND METHODS

### Study area

Sokoto state is located in the Sudan savannah zone in the extreme North-west part of Nigeria, between longitude 4° 8'E and 6° 5' E and latitude 12° 0' N and 13° 54'N (11). Rainfall in this area is between May/June to early October, when the natural water bodies are often flooded (Umar and Ipinjolu, 2010). Annual rainfall in that area ranges between 500 and 1300 mm, while the dry season last for 7 to 8 month that is October to May (SSMIYSC, 2001). It shared common borders with Niger Republic to the North, Kebbi state to the south-west and Zamfara state to the East. The total land area is about 32,000 square kilometers. The settlement areas in the district are mostly low lying with various types of fresh water bodies such as swamps, ponds and rivers. This area has two rivers that is, river Rima and river Sokoto. The vegetation is mainly grassland with trees. Kwakwalawa is a rural district around the river Rima, the district has mainly farmers and fishermen. People around the area are very poor and dependent on fish and other animals for food and nutrition and they use water from the river Rima for their domestic need.

### Sample collection

Urine and stool samples were collected mainly from school aged children because of their high risk of schistosomiasis infection. The infection status of this group gives a reliable reflection of the general

situation of the diseases in an area (Mafiana and Adesanya, 1999). Three schools were randomly selected in Sokoto, Sokoto State. In each selected school, 125 children were randomly screened for intestinal and urinary schistosomiasis, giving a total number of 375 samples of each urine and stool in the study area. For each child examined, a questionnaire aimed to determining the age, sex and water contact activity was provided for record. Urine samples were collected between the hours of 10:00 am to 2:00 pm and stool sample were collected in the morning since egg output from infected persons reaches at peak value around this time of the day (Grist et al., 1998; Rubin and Faber, 1999). Clean labelled specimen bottles were used for the collection of urine samples, small clean plastic cups with cover were used for stool collection. All the samples (Specimen bottles and plastic cups) were numbered such that they correspond with numbers of the subject on the questionnaire. All collected samples were taken to the Parasitological laboratory, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto and examined for *Schistosoma* infection. Stool samples were kept with 10% formalin solution for preservation while urine samples were preserved with 1% domestic bleach.

### Urine analysis

Urine samples were analyzed according to Pugh (1978) using a standard filtration technique. A 5.5 cm whatman's filter paper was inserted in the filtration unit. After shaking the urine sample, 10 ml of it was withdrawn with the help of a syringe and injected into filtration unit. After filtration, the filter paper was carefully removed using a pair of forceps and placed on a clean sheet of paper and stained with 50% ninhydrin solution and a drop of iodine. The stained filter paper was allowed to dry for about 15 min after which it was placed on a clean glass slide and observed systematically under the microscope at  $\times 10$  magnification. All the eggs were counted and the result was recorded as parasite load and expressed as number of eggs per 10 ml (number of egg/10 ml) of urine.

### Stool analysis

Stool analysis was carried out according to kato-katz thick faecal smear technique (Borda and Pellegrino, 1971). The stool samples were sieved using a plastic sieve of 0.75 mm pore size. A clean template was placed on a clean glass-slide with a spatula; the sieved stool specimen was used to fill the hole on the template. The template was removed leaving a plug of stool (about 50 mg) on the glass-slide one or two drops of 50% glycerol was added on the plug of stool and covered by cover slip, the whole preparation on the slide was then observed at lower magnification of  $\times 10$ , the result was systematically recorded and expressed as number of eggs per 50 mg of stool. The data was analysed by using analysis of variance (ANOVA) and Chi-Square to find out the similarities and differences between population and frequency.

## RESULTS

### Prevalence of urinary schistosomiasis

Table 1 showed that out of 375 samples of urine examined for *Schistosoma haematobium*, 228 (60.80%) were found infected with the parasite. However, the prevalence

**Table 1.** Prevalence of urinary schistosomiasis among the primary school's pupils in study area.

| Name of school                       | No. Examined | No. Positive | Prevalence (%) | Parasite load |
|--------------------------------------|--------------|--------------|----------------|---------------|
| Sultan Ibrahim Dasuki Primary School | 125          | 53.00        | 42.4           | 24.99         |
| Malamwa Primary School               | 125          | 81.00        | 64.8           | 43.86         |
| Basansan Model Primary School        | 125          | 94.00        | 75.2           | 45.49         |
| Total                                | 375          | 228.00       | 60.80          | 43.85         |

Prevalence is Calculated by No. of Positives/ No. of people examined  $\times 100$ . Parasite load- mean number of eggs/10 ml of urine.

**Table 2.** Age related prevalence of urinary schistosomiasis in the study area.

| Age group  | No. Examined | No. Positive | Prevalence (%) | Parasite load |
|------------|--------------|--------------|----------------|---------------|
| 5 – 9      | 100          | 43           | 43.00          | 23.83         |
| 9 – 12     | 175          | 125          | 71.42          | 50.93         |
| 13 – above | 100          | 60           | 60.00          | 43.86         |
| Total      | 375          | 228          | 60.80          | 43.85         |

Prevalence = No. of Positives/ No. of people examined  $\times 100$ . Parasite load- mean number of eggs/10 ml of urine.

**Table 3.** Sex related prevalence of urinary schistosomiasis in the study area.

| Sex    | No. Examined | No. Positive | No. Prevalence | Parasite load |
|--------|--------------|--------------|----------------|---------------|
| Male   | 235          | 187          | 79.57          | 45.45         |
| Female | 140          | 41           | 29.28          | 36.58         |

Prevalence = No. of Positives/ No. of people examined  $\times 100$ . Parasite load- mean number of eggs/10 ml of urine.

prevalence for the infection varied among the primary schools studied. Sultan Ibrahim Dasuki Primary School has the prevalence of 42.40% and parasite load with 24.99. Basansan Model Primary School and Malamawa Primary School had the highest prevalence of 75.20 and 64.80%, respectively, parasite load 45.49 and 43.86 were found in Basansan Model Primary School and Malamawa Primary School, respectively. A chi-square analysis showed significant variation ( $p < 0.01$ ) in the prevalence of the infection among the primary schools. Table 2 shows the age specific prevalence that persons within the age range of 9 to 12 years had the highest prevalence (71.42%) and parasite load of (50.93) followed by 13 years and above with 60.00 and 43.00%, for those below 8 years had the least prevalence and lowest parasite load of 23.83. Table 3 represents sex related prevalence of schistosomiasis. A total number of 235 males and 140 females urine examined showed a high significant variation ( $P < 0.01$ ) between the sex, when analysis of data was considered. However the highest prevalence rate and parasite load was recorded in male with (79.57%) and (45.45), respectively, compared to female with a

prevalence rate of 29.28% and parasite load of 35.58.

### Prevalence of intestinal schistosomiasis

Table 4 showed the prevalence of intestinal schistosomiasis among study area. Malamawa Primary School had the highest prevalence of 4.00%, followed by Basansan Model Primary School with prevalence of 3.2%, Sultan Ibrahim Dasuki Primary School had lowest prevalence of 2.93% intestinal schistosomiasis. Table 5 showed the prevalence of *S. mansoni* in relation to age group. 9 to 12 years old pupils had the highest prevalence of 4.2% intestinal schistosomiasis, followed by 5 to 8 years age group (2.54%) and 13 – above years old had the least prevalence of 2.17% while parasite load found at age group of 9 to 12 years of pupil is 1.80 and at the age group of 5 to 8 years old pupils with 1.60 and lastly 13 to above years of pupil is 1.66. However, a highly significant variation ( $P < 0.01$ ) was observed between the prevalence of the infection and age group of pupils in the study area. Table 6 shows the prevalence of



**Table 4.** Prevalence of intestinal schistosomiasis of pupils in the study area.

| Name of school                | No. Examined | No. Positive | Prevalence (%) | Parasite load |
|-------------------------------|--------------|--------------|----------------|---------------|
| Sult. Ibr. D. Primary School  | 125          | 2.00         | 160            | 1.40          |
| Malamawa Primary School       | 125          | 5.00         | 4.00           | 2.25          |
| Basansan Model Primary School | 125          | 4.00         | 3.20           | 1.15          |
| Total                         | 375          | 11           | 2.93           | 1.71          |

Prevalence = No. of Positives/ No. of people examined  $\times 100$ . Parasite load- mean number of eggs/50 mg of stool.

**Table 5.** Age related prevalence of intestinal schistosomiasis of pupils in primary schools.

| Age group (years) | No. Examined | No. Positive | Prevalence (%) | Parasite load |
|-------------------|--------------|--------------|----------------|---------------|
| 5 – 8             | 118          | 3            | 2.54           | 1.60          |
| 9 – 12            | 119          | 5            | 4.20           | 1.80          |
| 13 - above        | 138          | 3            | 2.17           | 1.66          |
| Total             | 375          | 11           | 2.93           | 1.72          |

Prevalence = No. of Positives/ No. of people examined  $\times 100$ . Parasite load- mean number of eggs/50 mg of stool.

**Table 6.** Sex related prevalence of intestinal schistosomiasis in the study area.

| Sex    | No. Examined | No. Positive | Prevalence (%) | Parasite load |
|--------|--------------|--------------|----------------|---------------|
| Male   | 189          | 7            | 3.80           | 7.22          |
| Female | 186          | 4            | 2.15           | 1.75          |
| Total  | 375          | 11           | 2.93           | 1.72          |

Prevalence = No. of Positives/ No. of people examined  $\times 100$ . Parasite load- mean number of eggs/50 mg of stool.

*S. mansoni* infection among sex, that is, males and females in the study area. Males were found more infected with prevalence of (3.80%) and females with prevalence of (2.15%), respectively. Parasite load found in males is 1.85 while in female is 1.75. Therefore, a high significant variation ( $P < 0.01$ ) among the sex was observed.

## DISCUSSION

It is clear from the results that the study area is endemic of urinary schistosomiasis and intestinal schistosomiasis. The prevalence rate of urinary schistosomiasis is (60.80%), and for the intestinal schistosomiasis prevalence rate is (2.93%), which may be attributed to water contact activities in the area as observed elsewhere (Abolarinwa, 1999; Agi and Okafor, 2005; Pukuma and Musa, 2007). The study area is rural communities, who depend on the ponds, wells, rivers, streams, dams and boreholes for their water needs such as drinking, farming (irrigation), bathing and other domestic uses. Most of

these water bodies are main transmission foci in the community and are distributed within the area. They provide a natural water sources and also serve as meeting point for the schistosome parasites. These ensure that the people continue to be infected and the re-infected since no intervention strategy has been carried out in the area. However, the variability found in the prevalence of the infection among the schools examined could be attributed to the fact that pupils living in rural area that is, Masanawa and Basansan depend on rivers, dams, pond and wells for their every day water demand, from where they got infection of disease.

The age specific prevalence of urinary schistosomiasis showed that pupils aged 9 to 12 years had the highest prevalence (71.42%), these pupils fall within the primary school age and in the villages this is the population most commonly found in prolonged water contact behaviour like swimming and playing, in bodies of water which are likely infested with infected snails. Other age groups of 13 to above years had an infection rate of 60.00% actively involved in such water contact behaviour as well as helping their parents in farming. This is in agreement with

results of reductions of eggs studies in other *S. haematobium* endemic areas (Adamu et al., 2001; Abdel-Wahab et al., 2000; Agi and Okafor, 2005; Ukpai and Ezeike, 2007; Pukuma and Musa, 2007; Bello et al., 2003).

In relation to sex, the high infection rate observed in males than in females was also observed in other endemic areas as found by other authors (Abolarinwa, 1999; Uwaezuoke et al., 2007; Ekejindu et al., 2002; Pukuma and Musa, 2007). This high prevalence in males than in females may be connected with the socio-cultural setup of the people of the study area. These people are predominantly Muslims, Hausa and Fulani by tribe. Majority of the females are restricted to their houses therefore they have less contact with infested water compared to their male counterparts. Swimming and bathing in the open water bodies is also very uncommon among females in community. This is in line with the observation made by other authors (Bello et al., 2003; Agi and Okafor, 2005).

## CONCLUSION AND RECOMMENDATIONS

Though the disease kills few people, its clinical effects, prevalence and association with other diseases and expansion of agriculture and water development projects, movement of population and increase in population density and some social habits like passing urine and faeces near water bodies makes it a problem of great health importance (WHO, 2010). The study therefore recommends the provision of pipe borne water in order to reduce their dependence on open water bodies for drinking and other domestic uses. The government should as a matter of policy institute a control programme in the form of hand washing campaigns, proper waste/faeces disposal and provision of pipe-borne water for schistosomiasis control in the affected areas in order to reduce its prevalence.

## Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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*Full Length Research Paper*

# Daytime sleepiness, circadian preference, caffeine consumption and use of other stimulants among Thai college students

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This study was conducted to evaluate the prevalence of daytime sleepiness and evening chronotype and to assess the extent to which both are associated with the use of caffeinated stimulants among 3,000 Thai college students. Demographic and behavioral characteristics were collected using a self-administered questionnaire. The Epworth Sleepiness Scale and the Horne and Ostberg Morningness-Eveningness Questionnaire were used to evaluate prevalence of daytime sleepiness and circadian preference. Multivariable logistic regression models were used to evaluate the association between sleep habits and consumption of caffeinated beverages. Overall, the prevalence of daytime sleepiness was 27.9% (95% CI: 26.2 to 29.5%) while the prevalence of evening chronotype was 13.0% (95% CI: 11.8 to 14.2%). Students who use energy drinks were more likely to be evening types. For instance, the use of M100/M150 energy drinks was associated with a more than 3-fold increased odds of evening chronotype (OR 3.50; 95% CI 1.90 to 6.44), while Red Bull users were more than twice as likely to have evening chronotype (OR 2.39; 95% CI 1.02 to 5.58). Additionally, those who consumed any energy drinks were more likely to be daytime sleepers. For example, Red Bull (OR 1.72; 95% CI 1.08 to 2.75) or M100/M150 (OR 1.52; 95% CI 1.10 to 2.11) consumption was associated with increased odds of daytime sleepiness. Our findings emphasize the importance of implementing educational and prevention programs targeted toward improving sleep hygiene and reducing the consumption of energy drinks among young adults.

**Key words:** Daytime sleepiness, Caffeine, stimulants, college students, morningness-eveningness.

## INTRODUCTION

Sleep is essential for maintaining good health in humans (Luyster et al., 2012). However, adolescents experience

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many changes in their sleep patterns in part due to physiological delayed sleep phase and disruption of the homeostatic sleep-wake cycle (Millman, 2005; Oginska and Pokorski, 2006). The challenges associated with the transition to college, including reduced parental supervision, extracurricular activities, academic workloads and social commitments (Malinauskas et al., 2007; Millman, 2005; Oginska et al., 2006) adversely affect sleep duration and sleep patterns of college students (Lund et al., 2010; Millman, 2005). Irregular sleep patterns can lead to daytime sleepiness, which is a result of sleep loss and deprivation (AASM, 2001; Kushida, 2006). There is a substantial body of evidence that shows daytime sleepiness is associated with reduced emotional intelligence, impaired constructive thinking skills, poor academic performance, job loss, headaches and obesity (AASM, 2001; Killgore et al., 2008; Slater et al., 2013).

Additionally, circadian rhythms and preferences can also be affected by social, biological and environmental factors (Besoluk et al., 2011; Kanerva et al., 2012; Lucassen et al., 2013). There are two major types of circadian preferences: morningness and eveningness (Horne and Ostberg, 1976; Schneider et al., 2011). Morning types tend to wake up relatively early in the morning and work best during this time (Schneider et al., 2011). On the other hand, evening types wake up later in the day and prefer to work during the evening and night (Schneider et al., 2011). An accumulating body of literature has confirmed that evening types experience higher sleep apnea, increased stress hormones, less healthier lifestyles and lower academic performances, although much of the research has focused on Western populations (Besoluk et al., 2011; Kanerva et al., 2012; Lucassen et al., 2013; Merikanto et al., 2013).

Caffeinated beverages and energy drinks have been implicated as important risk factors for increased daytime sleepiness and evening chronotype among college students (Ishak et al., 2012; Taillard et al., 1999). Globally, energy drinks have gained popularity among adolescents and young adults to counteract tiredness and meet academic, physical and cognitive demands (Buxton and Hagan, 2012; Malinauskas et al., 2007; Pomeranz et al., 2013; Roehrs and Roth, 2008). Some adverse effects of caffeine intake include energy loss, headaches, cardiac problems and even sudden death (Cannon et al., 2001; Roehrs and Roth, 2008; Seifert et al., 2011).

Despite the rising trends and aggressive marketing strategies aimed toward college students, there has been little research done on college students' intake of energy drinks and their impact on sleep disorders (Arria et al., 2010; Malinauskas et al., 2007). In particular, Thailand leads in consumption of energy drink consumption per capita globally, but very few studies have focused on sleep-related health problems in Southeast Asian populations (Cheng et al., 2012; Doi et al., 2003; Mak et al., 2010; Tsai and Li, 2004; Zenith International, 2007).

Given documented relationships between energy drinks

and sleep disorders, we hypothesized that students who use more stimulants were more likely than non-users to experience daytime sleepiness (Ishak et al., 2012; James et al., 2011; Roehrs and Roth 2008). We also hypothesized that students who use stimulants are more likely than non-users to be evening types (Taillard et al., 1999). From this study, we expect to provide evidence that will guide the development of health and wellness programs for young adults in Thailand.

## MATERIALS AND METHODS

### Study setting and sample

This cross-sectional study was conducted between December, 2010 and February, 2011 at seven private and government colleges in Thailand (Lohsoonthorn et al., 2012). Details of the study setting, sampling and data collection procedures have been described previously (Lohsoonthorn et al., 2012). Briefly, flyers were posted in each campus to recruit and invite participants to the study. Students who expressed an interest in participating were asked to meet in a large classroom or an auditorium where they were informed about the purpose of the study. Students consenting to participate were asked to complete a self-administered individual survey. Vision impaired students and those who could not read the consent and questionnaire forms were not eligible to participate. Those enrolled in correspondence, extension or night school programs were not included as well since their experience might be different from regular time students. Approximately 90% students invited to participate in the survey elected to do so. A total of 3,000 undergraduate students participated in the study and completed a self-administered survey. All study questionnaires were anonymous, and no personal identifiers were collected. Study procedures were approved by the institutional review boards of the Faculty of Medicine Chulalongkorn University, Walailak University and the University of Washington, USA. The Harvard School of Public Health Office of Human Research Administration, USA, granted approval to use the anonymous data set for analysis.

### Data collection and covariates

#### Demographics

In this study, a self-administered questionnaire was used to collect information on demographics and lifestyle characteristics. These include age, sex, education level, smoking status, physical activity, caffeine use, and alcohol consumption. The student's height, weight, waist and hip circumference were measured by research nurses after the questionnaire was administered.

#### Use of stimulant beverages and other caffeinated drinks

Participants were first asked if they consumed any stimulant or energy drink during the past week. Participants answering "Yes" were further asked to identify the specific types of energy drinks and/or stimulant drinks. These are beverages used to provide an extra boost in energy, promote wakefulness and provide cognitive and mood enhancement (Ishak et al., 2012). In this study, we will be using the terms energy drinks and stimulant beverages interchangeably. To provide a range of popular energy drinks in the immediate geographic regions where the survey was administered, we included examples of energy drinks that were common on the

campuses and in surrounding social establishments. These included global and local brands such as Red Bull, M100, M150, CarabaoDaeng, Lipovitan-D or Lipo, Wrangyer and Sharks. For the purpose of this analysis, we combined energy drinks that were less commonly used (that is, CarabaoDaeng, Lipovitan-D or Lipo, Wrangyer and Shark) and classified them as "other energy drinks". Consumption of other caffeine-containing beverages included coffee, black tea and stimulant beverages such as Coke and Pepsi or sugar-free Coke and Pepsi (contain about 35 mg of caffeine). Each caffeinated beverage was dichotomized (yes vs. no).

#### **Other covariates**

Alcohol consumption was defined as low (<1 alcoholic beverage a week), moderate (1 to 19 alcoholic beverages a week) and high to excessive consumption (>19 alcoholic beverages a week) (World Health Organization (WHO), 2004). Other covariates considered were: age (years), sex, cigarette smoking history (never, former, current) and participation in moderate or vigorous physical activity (no vs. yes); Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. BMI thresholds were set according to previously defined WHO cutoff points (underweight, <18.5 kg/m<sup>2</sup>; normal, 18.5 to 24.9 kg/m<sup>2</sup>; overweight, 25.0 to 29.9 kg/m<sup>2</sup>; and obese, 30 kg/m<sup>2</sup>) (WHO, 1995).

#### **Epworth sleepiness scale (ESS)**

The Epworth sleepiness scale (ESS) was used to evaluate general level of daytime sleepiness (Johns, 1991) and the capability to stay alert and awake during crucial moments of the day (AASM, 2001). The ESS is a brief instrument that has been widely used globally among different study populations. It has 8 items capturing an individual's propensity to fall asleep during commonly encountered situations, each measured on a scale from 0 to 3. The scores for the eight questions are added together to obtain a single total score that ranges from 0 to 24. In adults, an ESS score  $\geq 10$  is taken to indicate increased daytime sleepiness (Johns, 1991). In this study we used the ESS  $\geq 10$  cutoff point to define daytime sleepiness.

#### **The Horne and Ostberg morningness-eveningness questionnaire (MEQ)**

Circadian preference was assessed using the Horne and Ostberg morningness-eveningness questionnaire (Horne et al., 1976). Used globally, the MEQ (Horne et al., 1976) is a 19-item questionnaire that identifies morningness-eveningness preference (Merikanto et al., 2013; Schneider et al., 2011). Circadian rhythm is an individual's endogenous sleep-wake state during a 24-h period, while circadian preference refers to a person's inclination to sleep and engage in activities during that same period (Barion and Zee, 2007; Horne et al., 1976; Schneider et al., 2011). The scores range from 16 to 86 and participants can be classified in five categories: definite and moderate evening (E)-type, neutral type and moderate and definite morning (M)-type. Higher values on MEQ indicate stronger morningness preference. In this study we used the following cut offs: (1) 16 to 41 for evening; (2) 42 to 58 for intermediate; (3)  $\geq 59$  for morning. In this study, we excluded intermediate types from analysis when comparing morningness and eveningness chronotypes.

#### **Statistical analysis**

We first examined the frequency distributions of socio-demographic and behavioral characteristics of the study participants.

Characteristics were summarized using means ( $\pm$  standard deviation) for continuous variables with normal distribution and counts and percentages for categorical variables. Mean ( $\pm$  standard deviation) of MEQ and ESS scores were calculated across socio-demographic and behavioral characteristics and the associations were tested using a one-way ANOVA for multi-level characteristics and a two-sample t-test for two-level characteristics. Multivariable linear regression models were also fitted to evaluate the associations. We also calculated the distribution of morningness-eveningness chronotype across demographic and behavioral characteristics and Chi-square tests were used to determine bivariate differences. Additionally, we calculated the distribution of daytime sleepiness and evening chronotype across energy drink consumption status. Multivariable logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs) for the associations. All analysis were performed using SPSS Statistical Software for Windows (IBM SPSS, version 20, Chicago, IL, USA). All reported p-values are two-sided and deemed statistically significant at a 0.05 level.

## **RESULTS**

Of the 3,000 college students who completed the survey and met participant guidelines, 66.9% of the students were females and the average reported age was  $20.3 \pm 1.3$  years (Table 1). About two-thirds of students reported never drinking, while 32.1% admitted drinking 1 to 19 drinks per month; very few (1.7%) used alcohol excessively. A large majority of students have normal BMI (68.9%), few are overweight (10%) and 16.5% are underweight. Over three-fourths of study participants reported engaging in some type of physical activity. Table 2 shows the prevalence estimates of chronotype levels according to the Horne and Ostberg criteria. Thirteen percent of students were classified as evening types (12% in females and 15.1% in males) while 18.7% were classified as morning types (19.7% in females and 16.5% in males).

The prevalence of daytime sleepiness across age groups and sex is displayed in Figure 1. Daytime sleepiness (ESS  $\geq 10$ ) was present in 27.9% of the students (95% CI: 26.2 to 29.5%). Overall males in the 18 and the 22-and-over age groups appear to have higher prevalence of daytime sleepiness compared to females; 27.6% of the 18 year-old males experience daytime sleepiness compared to 21.2% of females; 35% of males 22-and-over experience daytime sleepiness compared to 27.4% of females. For the other age groups, the prevalence of daytime sleepiness is higher among females. Looking at the distribution of ESS total score across sex, the median ESS total score of females are higher than males.

As shown in Table 3, females (vs. males) and smokers (current and former vs. non-smokers) had a significantly lower MEQ score (p value < 0.001). There is also a significant association between MEQ score and alcohol consumption (p value < 0.001); we noted a trend lower MEQ scores with higher levels of alcohol consumption. Age, obesity status and participation in physical activity

**Table 1.** Characteristics of study sample.

| <b>Characteristic</b>                     | <b>N = 3,000</b> | <b>%</b> |
|---|------------------|----------|
| Age (Mean± SD)                            |                  | 20.3±1.3 |
| <b>Age (years)</b>                        |                  |          |
| 18  | 162              | 5.4      |
| 19  | 705              | 23.5     |
| 20  | 860              | 28.6     |
| 21  | 728              | 24.3     |
| ≥ 22                                      | 545              | 18.2     |
| <b>Sex</b>                                |                  |          |
| Female                                    | 2,008            | 66.9     |
| Male                                      | 992              | 33.1     |
| <b>Cigarette smoking status</b>           |                  |          |
| Never                                     | 2,739            | 91.3     |
| Former                                    | 55               | 1.8      |
| Current                                   | 206              | 6.9      |
| <b>Alcohol consumption</b>                |                  |          |
| <1 Drink/month                            | 1,986            | 66.2     |
| 1-19 Drinks/month                         | 962              | 32.1     |
| ≥ 20 Drinks/month                         | 52               | 1.7      |
| <b>Body mass index (kg/m<sup>2</sup>)</b> |                  |          |
| Underweight (<18.5)                       | 495              | 16.5     |
| Normal (18.5–24.9)                        | 2,068            | 68.9     |
| Overweight (25.0–29.9)                    | 298              | 10       |
| Obese (≥30.0)                             | 139              | 4.6      |
| <b>Any physical activity</b>              |                  |          |
| No  | 669              | 22.4     |
| Yes                                       | 2,319            | 77.6     |

**Table 2.** Prevalence estimates of morningness/eveningness chronotype

| <b>Parameter</b>       | <b>MEQ score cut-off</b> | <b>All</b>        | <b>Female</b>    | <b>Male</b>      |
|------------------------|--------------------------|-------------------|------------------|------------------|
|                        |                          | <b>% (95% CI)</b> |                  |                  |
| Evening type (n=378)   | ≤41                      | 13.0 (11.7-14.2)  | 12.0 (10.5-13.4) | 15.1 (12.8-17.3) |
| Intermediate (n=1,984) | 42-58                    | 68.3 (66.6-69.9)  | 68.2 (66.1-70.3) | 68.4 (65.4-71.3) |
| Morning type (n=544)   | ≥59                      | 18.7 (17.3-20.1)  | 19.7 (18.0-21.5) | 16.5 (14.1-18.9) |

were not found to be statistically significantly associated with MEQ scores. The multivariable linear regression model with all the demographic and lifestyle characteristics gave similar results, except that sex was no longer found to be significant (Table 1).

As shown in Table 4, females (vs. males), alcohol consumers (vs. non-consumers), physically active respondents

(vs. non-physically active respondents) and non-obese individuals (vs. obese individuals) had a significantly higher ESS score ( $p$  value < 0.037). Age and smoking status was not found to be significantly associated with ESS score. The results from the multivariable linear regression model were not appreciably different from the univariate analyses (Table 2).

**Table 3.** Morningness/eveningness questionnaire (MEQ) scores by demographic and lifestyle characteristics.

| Parameter                               | MEQ score |      | p-value |
|---|-----------|------|---------|
|   | Mean      | SD   |         |
| <b>Age (years)</b>                      |           |      |         |
| 18                                      | 50.70     | 8.28 | 0.241   |
| 19                                      | 51.66     | 8.02 |         |
| 20                                      | 50.87     | 8.09 |         |
| 21                                      | 50.75     | 8.06 |         |
| ≥ 22                                    | 50.97     | 8.44 |         |
| <b>Sex</b>                              |           |      |         |
| Female                                  | 51.45     | 8.07 | <0.001  |
| Male                                    | 50.19     | 8.23 |         |
| <b>Cigarette smoking status</b>         |           |      |         |
| Never                                   | 51.40     | 8.00 | <0.001  |
| Former                                  | 47.56     | 8.97 |         |
| Current                                 | 47.02     | 8.56 |         |
| <b>Alcohol consumption</b>              |           |      |         |
| <1 Drink/month                          | 52.14     | 8.07 | <0.001  |
| 1-19 Drinks/month                       | 49.04     | 7.75 |         |
| ≥ 20 Drinks/month                       | 45.31     | 8.76 |         |
| <b>Obesity (≥30.0 kg/m<sup>2</sup>)</b> |           |      |         |
| No                                      | 51.07     | 8.15 | 0.334   |
| Yes                                     | 50.38     | 7.99 |         |
| <b>Any physical activity</b>            |           |      |         |
| No                                      | 51.20     | 8.23 | 0.614   |
| Yes                                     | 51.01     | 8.11 |         |

**Table 4.** Epworth sleepiness scale (ESS) scores by demographic and lifestyle characteristics.

| Parameter                               | EES score |      | p-value |
|---|-----------|------|---------|
|   | Mean      | SD   |         |
| <b>Age (years)</b>                      |           |      |         |
| 18                                      | 7.44      | 2.84 | 0.635   |
| 19                                      | 7.56      | 3.35 |         |
| 20                                      | 7.54      | 3.33 |         |
| 21                                      | 7.57      | 3.47 |         |
| ≥ 22                                    | 7.79      | 3.52 |         |
| <b>Sex</b>                              |           |      |         |
| Female                                  | 7.68      | 3.32 | 0.037   |
| Male                                    | 7.40      | 3.50 |         |
| <b>Cigarette smoking status</b>         |           |      |         |
| Never                                   | 7.54      | 3.36 | 0.059   |
| Former                                  | 8.11      | 3.53 |         |
| Current                                 | 8.05      | 3.56 |         |
| <b>Alcohol consumption</b>              |           |      |         |
| <1 drink/month                          | 7.46      | 3.39 | 0.009   |
| 1-19 drinks/month                       | 7.84      | 3.32 |         |
| ≥ 20 drinks/month                       | 8.12      | 3.65 |         |
| <b>Obesity (≥30.0 kg/m<sup>2</sup>)</b> |           |      |         |
| No                                      | 7.62      | 3.39 | 0.165   |
| Yes                                     | 7.38      | 3.31 |         |
| <b>Any physical activity</b>            |           |      |         |
| No                                      | 7.18      | 3.45 | 0.001   |
| Yes                                     | 7.70      | 3.34 |         |

Table 5 summarizes the logistic regression results. Consumers of any stimulant beverage had 2.68-folds higher odds of being evening chronotypes compared to those who abstained from consuming stimulant beverages (OR 2.68; 95% CI 2.01 to 3.58), after adjusting for age, sex, smoking, BMI and physical activity. When considering specific types of beverages, the odds of being evening chronotype were between 1.95- and 3.5-fold higher among users compared to non-users. Compared to those who consumed less than one stimulant beverages per week, those who consumed two per week have 2.65-folds higher odds of being evening chronotypes (OR 2.65; 95% CI 1.81 to 3.90) while those who consumed three or more have 3.65-folds higher odds of being evening chronotypes (OR 3.65; 95% CI 2.58 to 5.16). However, consuming one stimulant per week was not statistically significantly associated with the odds of being evening chronotypes (OR 1.41; 95% CI .87 to 2.29). Con-sumers of stimulant beverages had a 22%

higher odds of experiencing daytime sleepiness compared to non-consumers (OR 1.22; 95% CI 1.03 to 1.44), after adjusting for demographic and lifestyle characteristics. When considering specific types of beverages, the odds of experiencing daytime sleepiness were between 1.21- and 1.72-fold higher among users compared to non-users; exceptions included coffee drinkers or consumers of other types of energy drinks, for whom no statistically significant association were found. Those who consumed three or more stimulants per week had 37% higher odds of experiencing daytime sleepiness compared to those who do not use stimulants (OR 1.37; 95% CI 1.13 to 1.67).

## DISCUSSION

To the best of our knowledge, this is the first study to examine the prevalence of daytime sleepiness and evening

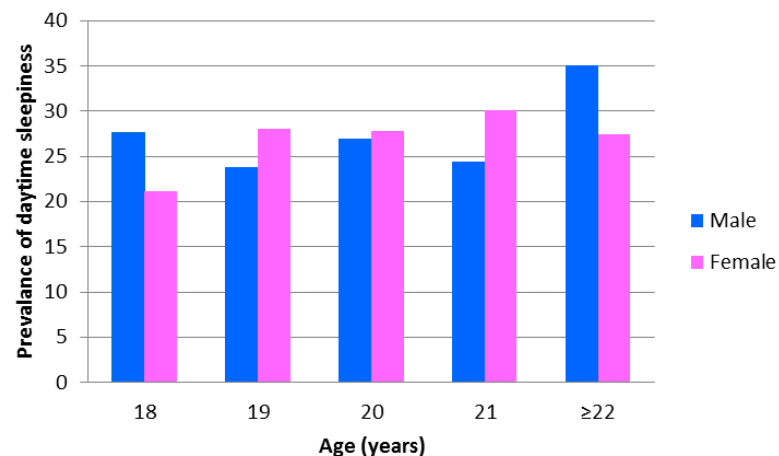


Figure 1. Prevalence of daytime sleepiness by age and sex.

Table 5. Evening chronotype and daytime sleepiness in relation to stimulant use.

| Parameter   | Evening Chronotype |            |                        |                       | Daytime Sleepiness |              |                        |                       |
|---|--------------------|------------|------------------------|-----------------------|--------------------|--------------|------------------------|-----------------------|
|   | Yes (N=378)        | No (N=544) | Unadjusted OR (95% CI) | Adjusted* OR (95% CI) | Yes (N=831)        | No (N=2,152) | Unadjusted OR (95% CI) | Adjusted* OR (95% CI) |
| <b>Any stimulant beverages</b>                      | %                  | %          |                        |                       | %                  | %            |                        |                       |
| No  | 29.6               | 54.6       | 1.00 (Reference)       | 1.00 (Reference)      | 38.6               | 43.7         | 1.00 (Reference)       | 1.00 (Reference)      |
| Yes   | 70.4               | 45.4       | 2.86 (2.16-3.77)       | 2.68 (2.01,3.58)      | 61.4               | 56.3         | 1.24 (1.05-1.45)       | 1.22 (1.03-1.44)      |
| <b>Type of beverage</b>                             |                    |            |                        |                       |                    |              |                        |                       |
| Coffee  | 32.5               | 19.1       | 2.04 (1.51-2.76)       | 1.95 (1.42-2.67)      | 26.2               | 25.0         | 1.07 (0.89-1.28)       | 1.07 (0.89-1.29)      |
| Tea   | 57.4               | 37.1       | 2.28 (1.75-2.98)       | 2.31 (1.75-3.05)      | 51.3               | 46.1         | 1.23 (1.05-1.45)       | 1.21 (1.03-1.43)      |
| Coke/Pepsi with sugar                               | 57.1               | 31.4       | 2.91 (2.22-3.82)       | 2.70 (2.03-3.58)      | 49.2               | 43.1         | 1.28 (1.09-1.50)       | 1.26 (1.07-1.48)      |
| Coke/Pepsi sugar free                               | 19.8               | 8.3        | 2.75 (1.847-4.08)      | 2.66 (1.77-4.00)      | 14.7               | 11.1         | 1.38 (1.09-1.74)       | 1.39 (1.10-1.76)      |
| M 100/M 150   | 11.6               | 2.9        | 4.35 (2.41-7.83)       | 3.50 (1.90-6.44)      | 7.9                | 5.3          | 1.56 (1.14-2.13)       | 1.52 (1.10-2.11)      |
| Red Bull  | 4.8                | 1.7        | 2.97 (1.32-6.69)       | 2.39 (1.02-5.58)      | 3.9                | 2.2          | 1.76 (1.11-2.77)       | 1.72 (1.08-2.75)      |
| Other Energy Drinks**                               | 3.4                | 0.9        | 3.84 (1.36-10.86)      | 2.90 (0.98-8.62)      | 2.8                | 1.6          | 1.72 (1.01-2.93)       | 1.67 (0.97-2.88)      |
| <b>Number of different types of stimulants/week</b> |                    |            |                        |                       |                    |              |                        |                       |
| 0   | 29.6               | 54.6       | 1.00 (Reference)       | 1.00 (Reference)      | 38.6               | 43.7         | 1.00 (Reference)       | 1.00 (Reference)      |
| 1   | 8.5                | 11.0       | 1.41 (0.87-2.29)       | 1.21 (0.73-2.00)      | 8.8                | 9.5          | 1.04 (0.78-1.40)       | 1.03 (0.76-1.39)      |
| 2   | 22.2               | 15.3       | 2.68 (1.85-3.90)       | 2.65 (1.81-3.90)      | 18.5               | 18.8         | 1.12 (0.89-1.40)       | 1.10 (0.87-1.38)      |
| ≥3  | 39.7               | 19.1       | 3.83 (2.75-5.327)      | 3.65 (2.58-5.16)      | 34.1               | 27.9         | 1.38 (1.14-1.67)       | 1.37 (1.13-1.67)      |

\*Adjusted for age, sex, smoking, body mass index, and physical activity; \*\*Other energy drinks includes the following: CarabaoDaeng, Lipovitan-D or Lipo, Wrangyer and Shark.



evening chronotype in relation to caffeine use in a Southeast Asian population. Approximately 28% experienced daytime sleepiness while 13% of our student cohort reported to be evening types. Overall, students with daytime sleepiness were more likely to be cigarette smokers, alcohol drinkers, obese, physically active and stimulant beverage consumers. The odds of being evening types were elevated among cigarette smokers, alcohol consumers and caffeinated stimulant users.

Our results are in accordance with previous reports indicating prevalence of daytime sleepiness and eveningness chronotype and extend this literature to assess their associations with consumption of stimulant beverages in Thai young adults (Adan and Natale, 2002; Besoluk et al., 2011; Chung and Cheung, 2008; Hsu et al., 2012; Lund et al., 2010; Schneider et al., 2011; Wu et al., 2012; Yang et al., 2003). For instance, our result showing that 13% of Thai college students were evening types is consistent with reports from Chung and Cheung (2008) who reported a 13.1% prevalence of evening chronotype among Chinese students. Additionally, our findings showing a 27.9% daytime sleepiness are similar to those of Wu et al. (2012) who reported a 22.2% prevalence of daytime sleepiness. Other investigators, however, have reported higher prevalence estimates (Pirralo et al., 2012). On balance, the results of our study and those of others emphasize the growing problem of daytime sleepiness and eveningness chronotype among college students.

In our study of Thai college students, consuming any stimulant beverage was strongly associated with evening chronotype and daytime sleepiness. Prior studies have found similar results in other university settings (Giannotti et al., 2002; James et al., 2011; Roehrs and Roth 2008; Snel and Lorist, 2011; Taylor et al., 2011). College marks the beginning of new and stressful changes: increased academic workload, busier social lives and later bed times (Lund et al., 2010; Malinauskas et al., 2007). Several investigators determined that academic stress interferes with sleeping schedule, as students may stay up late into the night to study (Chung et al., 2008; Lund et al., 2010). Given the high prevalence of poor sleep quality, short sleep duration and high rates of energy drink consumption among Thai college students, (Steptoe et al., 2006; Zenith International, 2007) it may be fitting to note that these undergraduates may sacrifice sleep for academic purposes and social commitments and drink caffeinated stimulants to remain alert (Ishak et al., 2012; Malinauskas et al., 2007; Roehrs and Roth 2008). As they stay up later to finish their work, they can become sleep deprived due to the morning-oriented schedules of college and thus experience daytime sleepiness (Kushida, 2006; Wittmann et al., 2010).

Several investigators have noted that evening types and daytime sleepers are more likely to have poor health (AASM, 2001; Kanerva et al., 2012; Killgore et al., 2008; Nakade et al., 2009; Schneider et al., 2011; Slater et al.,

2013; Stroe et al., 2010; Wittmann et al., 2010). Our findings suggest that caffeine consumers were more likely to experience daytime sleepiness and be evening types. As a result, caffeine could possibly be linked to other poor health behaviors. Future research must evaluate the extent to which caffeinated beverages and energy drinks are associated with poor health traits.

The association between caffeinated drinks and circadian disruption and daytime sleepiness can be explained by the biological mechanism of melatonin suppression and adenosine blockage. During waking hours, light is known to suppress melatonin production (Barion et al., 2007; Gooley et al., 2011). Given the demanding college workload and social commitments, students may stay up later into the night to study, which requires the use of lighting and encourages the consumption of caffeinated beverages to increase alertness. This increased light exposure leads to melatonin suppression, in which light exposure at night shifts one's chronotype towards eveningness (Barion et al., 2007; Shanahan et al., 1999). Another mechanism that can explain sleep disruption is caffeine's role in adenosine blockage. Within the basal forebrain, adenosine, an endogenous biochemical compound, regulates sleep by inhibiting the cholinergic neurons that create arousal (Basheer et al., 2004; Ribeiro and Sebastiao, 2010; Roehrs and Roth 2008). As caffeine acts as an adenosine blocker, it negates the effects of adenosine to induce sleep (Roehrs and Roth 2008). Consequently, consuming caffeine at night prolongs wakefulness and decreases sleep duration, resulting in daytime sleepiness (James et al., 2011; Kushida, 2006; Roehrs and Roth 2008).

The results from our study should be interpreted in the context of some limitations. First, our study could be subjected to volunteer bias, because the data were collected from willing participants instead of a random sample. Second, the temporal relationship between lifestyle characteristics and sleep disorders cannot be delineated due to the cross-sectional study design. It is possible that daytime sleepiness and eveningness chronotype might have led to increased consumption of energy drinks and other caffeinated beverages. Prospective studies are required to confirm and expand upon our observations. Third, there may be lifestyle and dietary traits that are heterogeneous within daytime sleepers and evening types, which could affect the strength of the associations between sleep disorders and lifestyle traits. Fourth, our study population was exclusively comprised of participants who were attending college, thus conclusions cannot be other broader populations. Lastly, we did not have information concerning frequency, timing and dose of energy drink consumption in the present study. As a result, it is possible that the binary grouping of energy drink consumption may have attenuated the magnitude of the association toward the null.

## Conclusion

In summary, our findings underline the growing problem of energy drinks and their impact on both circadian preference and daytime sleepiness among Thai college students. Despite academics being the frequent reason given for energy drink consumption, evening types or daytime sleepers do not exhibit higher academic performance compared to their counterparts (Besoluk et al., 2011; Chung et al., 2008; Giannotti et al., 2002; James et al., 2011; Taylor et al., 2011).

## RECOMMENDATION

There is a large body of evidence that daytime sleepers and evening types are strongly associated with numerous health and social problems (AASM, 2001; Kanerva et al., 2012; Killgore et al., 2008; Nakade et al., 2009; Schneider et al., 2011; Slater et al., 2013). From a public health promotion and disease prevention standpoint, these findings suggest an obvious need for effective educational and prevention programs targeted toward improving sleep hygiene and reducing consumption of energy drinks among young adults. Future research that evaluates the impact of caffeinated beverages on various sleep disorders and those that assess the effectiveness of health and wellness programs among young adults, are needed.

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## Conflict of interest

The authors have no competing interests to declare.

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