Effect of circadian rhythm type on serum lipid levels in shift workers: 5-year cohort study

（交代勤務従事者の概日リズムタイプが血清脂質レベルに与える影響：コホート研究）

千葉大学大学院医学薬学府
先端医学薬学専攻
（主任：安西　尚彦　教授）
富澤　亜樹

ABSTRACT

Objective: We investigated how differences in circadian rhythm type affect the health of workers engaged in shift work.

Methods: Employees who were newly hired in a steel company between 2007 and 2011, received the Morningness - Eveningness Questionnaire (MEQ) survey. The target participants were 153 male shift workers who were not being treated with any antihyperlipidemic drugs and underwent periodic physical examinations including blood tests at least twice. According to the score of the MEQ at the time of joining the company, we classified the subjects into 5 types. Longitudinal changes in serum lipid level were estimated among the circadian rhythm types adjusted for age, BMI, and other covariates using a linear mixed model.

Results: The regression coefficient of total cholesterol level in the “definitely and moderately morning” group was -17.83 (95% confidence interval (CI):-33.42~-2.23), and in the "intermediate "group was -16.84 [95%CI:-30.40~-3.28], compared to the moderate evening type. The total cholesterol level was higher in the moderately evening type than in any of the other groups. Between the ME type and LDL cholesterol levels, compared with the "moderately evening type" group, the regression coefficient in the "intermediate type" group was -16.08 (95%CI:-28.79~-3.37), and in the "definitely and moderately morning type" group was -17.50 [95%CI:-32.11~-2.88]. The "moderately evening type" group had a higher LDL cholesterol level than any of the other groups.

Conclusions: Evening-type circadian rhythm type shift workers are more prone to elevated serum lipid levels.

Keywords: circadian rhythm type, shift work, Morningness - Eveningness Questionnaire, serum lipid level, longitudinal study

Introduction

Shift work has been adopted in most industries according to changes in the working environment. It has been suggested that shift work independently affects the onset of various diseases such as dyslipidemia, hypertension, coronary artery disease, and diabetes mellitus.

One reason why shift work affects health is believed to be the effect of shift work on the circadian rhythm which is a physiological and psychological parameter that is controlled by the hypothalamus[1]. Human beings have a biological circadian rhythm which is affected by light, environment, and lifestyle. In addition, each individual has his or her own rhythm.

As a method of evaluating these individual differences in circadian rhythms, research has been conducted using the Morningness - Eveningness Questionnaire (MEQ)[2]. MEQ is composed of 19 question items, and classifies people into five types: definite morning type, moderate morning type, intermediate type, moderate evening type, and definite evening type.

Morning type people wake up naturally in the early hours, and prefer to be active in the morning. A previous study[2] indicated that they feel drowsiness and fatigue early, and so go to bed early. On the other hand, evening type people wake up later in the day and prefer to be active in the afternoon and evening. Drowsiness and fatigue appear late at night, and so bedtime is delayed.

In many studies using MEQ, the evening type has been considered to be a risk factor that is undesirable both physically and mentally compared to the morning type[3 4]. Especially with regard to serum lipid levels, a previous study indicated that evening chronotype is related to higher triglyceride and lower HDL cholesterol levels[5]. However, hitherto no research has investigated how differences in circadian rhythm type affect the serum lipid levels of shift workers.

This prompted us to examine the effect of circadian rhythm type on serum lipid levels of Japanese male workers engaged in shift work.

METHODS

Subjects

The target cohort of this study was obtained from a database of annual health checkups for male workers engaged in shift work at a Japanese steel company.

Health checkups are held every year for all workers, with more than 98% of this company's employees participating.

For 512 employees who entered between 2007 and 2011, MEQ survey was conducted during the health checkup conducted at company entry. After the first health check up at entry to this company, 406 employees were engaged in shift work at the time of the second health checkup. We consider that the serial changes in employees could be evaluated with two or more examinations. Then, we targeted 153 people who had undergone annual health checkups and blood collection at least twice after company entry and who did not begin oral intake of any lipid therapeutic drugs.

The study protocol was approved by the Ethics Review Board of the Graduate School of Medicine, Chiba University.

Measurements

We classified the subjects based on the score of MEQ at entrance to the company as "definitely morning type" (70-86 points), "moderately morning type" (59-69 points), "intermediate type" (42-58 points), "moderately evening type" (31-41 points), "definitely evening type" (16-30 points)[2]. Because the number of the "definitely morning type" was extremely small, the "definitely morning type" group was combined with the "moderately morning type" group. This morning type group and the "intermediate group" were compared with the "moderately evening type" group. No "definitely night type" person was identified among the subjects.

The following data were collected by annual health checkup: serum lipids, age, BMI, medical history, drinking and smoking behaviors, physical activity, and job-related stress. Health checkups, including blood sampling, were carried out between 9 a.m. and 3 p.m. throughout the study period. None of the measurements were taken within 30 min of a meal. Body weight and height were measured at the same time, in light indoor clothing without shoes, using a fully automatic weighing machine. The laboratory tests were conducted in comprehensive clinical testing laboratories that had been guaranteed by an official certification organization. There was no change in the laboratory test methodology that required conversion due to significant differences.

The medical history, drinking, and smoking behaviors and physical activity of each worker were recorded during the annual health checkups using a self-administered questionnaire. The questionnaires were distributed to the employees as part of legally required annual health checkups for workers, and filled out before the health checkup. The completed questionnaires were then collected directly from the workers by the healthcare administration medical staff at the site of the health checkup. Coworkers of the subjects were therefore unaware of how the subjects had answered the questionnaire.

At the start of follow-up, a self-administered questionnaire prepared originally by an occupational physician for assessing the health maintenance of workers was used. This questionnaire was based on the conditions and medical needs in the workplace reported in previous studies [6 7].

The quantity of alcohol in each type of alcoholic beverage was calculated using ‘gou’, the most common unit in Japan for measuring alcohol consumption: 1 gou (180 mL) of Japanese sake (rice wine) contains approximately 22 g of ethanol, and is equivalent to 500 mL of beer, 60 mL of whiskey, 180 mL of wine and 110 mL of shochu (white spirits). This unit was used in the questionnaire as it was easily comprehensible by the general Japanese population for

determining the amount of alcohol in the beverages consumed. Weekly alcohol intake was estimated by multiplying quantity by frequency. Smoking status was classified as nonsmoker, 1-10 cigarettes/day, 11-20 cigarettes/day, 21-40 cigarettes/day, and 41 or more cigarettes/day. Physical activity behaviors were categorized as none, 1–2 times/month, 1–2 times/week, 3–4 times/week or ≥ 5 times/week.

As a survey of job-related stress, we used the Brief Job Stress Questionnaire (BJSQ) [8], which has been widely used in Japan, due to its reliability and validity.

BJSQ consists of the following items: job demands and control, interpersonal relationships, and support of superiors/colleagues.

We analyzed for each group corresponding to "high demands of job", "low control of job", "poor interpersonal relationships", and "little support of superiors/colleagues".

Statistical Analysis

We investigated the relationship between morning - evening type and serum total cholesterol, HDL-cholesterol and LDL-cholesterol levels. We analyzed the time course of physiological and laboratory data using a linear mixed model[9]. For each outcome variable, a model with the duration of shift work from the first health checkup at entry to this company, as the repeated measure factor, was constructed. MEQ category was included in the model as a fixed effect. Age at entry to this company, BMI, drinking behavior, physical activity and job-related stress were included as covariates. Subjects' ID was included as a random effect to account for the variability due to individual differences between subjects. Based on the obtained model, the average values of serum lipid levels of each MEQ group were estimated. The Holm method was used to adjust the P values in multiple testing.

The analyses were performed with IBM SPSS 19.0.2J (IBM Business Analytics, Tokyo). A P value<0.05 was considered statistically significant.

RESULTS

Table 1 shows the number of people by ME type at the time of the health checkup when the subjects entered the company. The average follow-up period was 4.3 years (1.8 to 6.6 years), the average age at company entry was 28.2 years, and the average BMI was 23.5. No participant was classified as being of the definitely evening type. Definitely and moderately morning groups were combined due to the small numbers in these groups.

Table 2 shows the regression coefficients for the increase in total cholesterol estimated using the linear mixed model. Using the “moderately evening type” as the control group for comparison, the regression coefficient of total cholesterol level in the “intermediate type” was -17.83 (95% confidence interval (CI): - 33.42 ~ -2.23), and in the "definitely and moderately morning type " was -16.84 [95%CI: -30.40 ~ -3.28], which was shown to be lower than the "moderately evening type".

No significant results were obtained regarding the relation between ME type and HDL cholesterol level (Table 3).

Between the ME type and LDL cholesterol levels, compared with the "moderately evening type", the regression coefficient in the "intermediate type" was -16.08 (95% CI: - 28.79 ~ -3.37), and in the "definitely and moderately morning type" was -17.50 [95% CI: -32.11 ~ -2.88], which was shown to be lower than the "moderately evening type" (Table 4).

Table 5 shows the estimated average values of serum lipid levels for each ME type based on the obtained statistical model. Total cholesterol level increased in the “moderately evening type” (198.5 mg / dL, 180.7 mg / dL in the “definitely / moderately morning type” group, 181.7 mg / dL in the “intermediate type” group). There was no significant difference in HDL cholesterol (56.0 mg / dL in the “moderately evening type” group to 57.1 mg / dL in the “intermediate type” group)). LDL cholesterol increased in the “evening type” group (124.5

mg / dL, 107.0 mg / dL in the “definitely / moderately morning type” group, 108.4 mg / dL in the “intermediate type” group).

For the other factors besides ME type (Table 2-4), total cholesterol and LDL cholesterol increased with age (2.15 [95% CI: -1.08 ~ 3.23], 1.23 [95% CI: 0.22 ~ 2.23]). As BMI increased, total cholesterol and LDL cholesterol increased (4.48 [95% CI: 3.37 ~ 5.59], 4.54 [95% CI: 3.52 ~ 5.56]), while HDL cholesterol decreased (-1.38 [95% CI: -1.80 ~ -0.96]). Depending on the amount of alcohol consumption, HDL cholesterol increased (0.60 [95% CI: 0.38 ~ 0.81]). HDL cholesterol was decreased in smokers compared with nonsmokers. The regression coefficient was -5.34 [95% CI: -7.65 ~ -3.04] in the "1-10 cigarettes/day" group, -4.82 [95% CI: -6.95 ~ -2.69] in the "11-20 cigarettes/day" group, and -4.24 [95% CI: -7.42 ~ -1.07] in the “21 or more cigarettes/day” group. No significant results were obtained showing any effects of exercise on serum lipid levels.

DISCUSSION

In the present study, total cholesterol and LDL cholesterol were significantly and apparently increased in the evening type compared to other ME types, after adjusting for other potential covariates. The present results indicated that the effect of shift work on workers’ health was modified by individual ME type.

The strength of the present study design lies in its longitudinal investigation of a single cohort. Furthermore, the study adopted a linear mixed model that adjusted for various confounding factors such as lifestyle, which were measured annually. This facilitated a more accurate calculation of the changes in repeatedly measured data. In the linear mixed model, each subject was assumed to have an underlying level of response, incorporated by regarding this subject effect as random [9]. We think that the linear mixed model could compensate for the absence of detailed data on lifestyle to some extent by taking this random subject effect

into consideration.

In addition, the physical examinations and laboratory tests were conducted as part of a comprehensive health examination carried out at a clinical testing center guaranteed by an official certification organization. As there were no changes in laboratory testing methodology that required conversion due to significant differences, we consider our findings not to have been attributable to either a flawed design or systematic measurement errors. The present new finding may be due to more precise identification of periods for continuation of shift work and the improved design of the present study. We therefore consider that these new comprehensive findings will be very informative for health guidance regarding serum lipid level control.

In this study, we examined the effect of different circadian rhythms on shift workers' health taking other potential covariates into account. This time, we focused on serum lipid levels. As far as we know, such evaluation has not been reported previously. Although some research on shift work and serum lipid levels has been conducted thus far[10 11], our results showed that serum lipid levels in shift workers tended to increase in the evening-type group more than in the morning-type group.

Circadian rhythms are determined by genes such as BMAL1 and CLOCK. Woon et al. found that polymorphisms in these genes are related to the prevalence of metabolic diseases in humans [12 13]. In animal experiments, it is reported that abnormalities in these genes may lead to dyslipidemia, insufficient insulin secretion and obesity [14 15]. According to a systematic review, shift workers are reported to have more frequent meals and malnutrition[16]. In terms of morning-eveningness type, Sato-Mito et al. reported that intake of alcohol, fat, meat, noodles and confectionery increased in evening type people compared to morning type ones [17]. Therefore, the eating habits of 'evening type', which may promote abnormal lipid metabolism, have been shown to be affected by shift work.

Several studies have reported that shift work is associated with elevated serum triglycerides, decreased serum HDL cholesterol, and increased serum total cholesterol levels [10 11 18-20]. In this study, we obtained new findings by examining serum lipids for differences in the health of shift workers considering circadian rhythm type.

In studies on circadian rhythm using the MEQ, being an evening type person is more often mentioned as a risk factor. Evening type is more likely to show severe depressive symptoms compared to morning type[3], while morning type is associated with good physical and mental health, self-affirmation, and family relationships[4]. Wong et al. indicated that evening chronotype is related to higher serum triglycerides and lower HDL cholesterol level [5]. In our study, it was also found that the health of evening type workers is more likely to be affected than that of morning type by shift work. In addition, age, BMI and smoking habits were associated with worsening serum lipid levels, while alcohol consumption was related to elevated HDL cholesterol.

As a limit of this research, in addition to the small sample size, female shift workers were excluded because of the small numbers available. On the other hand, Karlsson et al. reported that triglyceride significantly increased in both men and women in a previous study not including the analysis of circadian rhythm [18].

The average age of the subjects was young at 28.2 years. However, in previous studies, it has been reported that shift work increases the risk of dyslipidemia over a wide range of ages [18 19]. Furthermore in this study, we focused on serum lipid levels, and are planning to continue a further longitudinal analysis, focusing on parameters such as blood pressure, blood glucose, and uric acid levels.

Conclusion

In the present study, shift workers with evening-type circadian rhythm type were found to be more prone to elevated serum lipid levels. We believe that we can provide a more effective

occupational health service for shift workers which is suitable for individuals by also taking into account the physiological and psychological aspects of morning and evening type.

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