Relationship of shift work with liver enzyme abnormalities and nonalcoholic fatty liver disease: A retrospective cohort study of Thai workers in Bangkok, Thailand

Napas Taechasan[1], Wiroj Jiamjarasrangsi[1*]

[1]Health Research and Management Program, Department of Preventive and Social Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Corresponding Author: Wiroj Jiamjarasrangsi **Email:** wjiamja@gmail.com

ABSTRACT

Shift work, particularly night shift work, has several detrimental health outcomes. This retrospective study investigated the associations of shift work with the incidence rates of liver enzyme abnormalities and nonalcoholic fatty liver disease (NAFLD) using the annual health check-up database between 2009 and 2016 and self-administered questionnaires from two large organizations in Thailand. Among 10,205 participants, 3620 eligible participants, classified into three subgroups by their shift work status, were followed up until abnormal liver outcomes were observed. The incidence rate of abnormal outcomes was calculated, and Cox proportional hazard models were used to assess the associations. Current shift work was not associated with abnormal liver outcomes, including elevated alanine aminotransferase and aspartate aminotransferase levels, elevated lipid accumulation product (e-LAP), and elevated hepatic steatosis index (e-HSI). However, when the frequencies and duration of shift work were investigated, the duration of shift work was associated with NAFLD outcomes. Healthcare personnel with 10–20 years of shift work experience had a higher risk of e-LAP (adjusted hazard ratio [HR] = 1.31, 95% confidence interval [CI] = 1.04–1.65), while workers with more than 20 years of shift work had a lower risk of e-LAP (adjusted HR = 0.77, 95% CI–0.62–0.97). Nonhealthcare shift workers with fewer than 10 years of work exposure had a higher risk of e-HSI (adjusted HR = 4.30, 95% CI = 1.28–14.47). In summary, the shift work status was not associated with liver abnormalities, but the duration of shift work was associated with NAFLD.

Key words:
shift work; liver enzymes; nonalcoholic fatty liver disease; retrospective cohort study; Cox proportional hazard models

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a common chronic liver disease, and its global prevalence has increased from 25% to 38% over the past 30 years.[1] The spectrum of NAFLD ranges from reversible steatosis, nonalcoholic steatohepatitis (NASH) accompanied by abnormal liver enzyme levels, including elevated alanine aminotransferase (e-ALT) and elevated aspartate aminotransferase (e-AST) levels, to decompensated cirrhosis and liver cancer, and NAFLD accounts for 6%–17% of liver cancer related-deaths globally.[1-4] In addition, NAFLD, particularly NASH, carries a significant economic burden, including healthcare resource consumption and direct and indirect expenses.[5]

At present, the gold standard for NAFLD diagnosis is liver biopsy. However, it is invasive and expensive.[6] Thus, abdominal ultrasound is widely utilized as a noninvasive tool for NAFLD screening. However, the results are operator-dependent, and the test is time- and cost-consuming for large-scale screening.[6] Recent studies have developed several diagnostic scores for NAFLD using anthropometric and biochemical data, including the fatty liver index (FLI), NAFLD liver fat score, lipid accumulation product (LAP), and hepatic steatosis index (HSI). [7-10] These scores, particularly LAP and HSI, which have been confirmed by radiologic imaging in the general Asian adult population, have emerged as alterative noninvasive NAFLD screening tools.[10,11]

The substantial mechanism underlying NAFLD is metabolic imbalance in the liver, a vital organ involved in energy homeostasis and the detoxification of xenobiotics.[12] The working cycle of the liver is regulated by the biological clock, which has light as the key determinant, and this cycle can be disturbed in shift workers, who live against the natural circadian rhythm.[13,14]

Currently, approximately 15%–36% of workers globally are shift workers who work outside normal working hours, including at night.[15] According to Thai labor statistics, the exact proportion of shift workers is unknown, but it can be estimated from common job sectors with shift work schedules including healthcare, utilities, accommodation and food service, manufacturing, and transportation. The number of such jobs in Thailand increased from 13.5 million in 2016 to 14.1 million in 2022, representing approximately 5% of all workers.[16,17]

Previous studies suggested an association of shift work with abnormal liver outcomes including e-ALT and NAFLD investigated by abdominal ultrasound rather than NAFLD diagnostic scores.[18-20] Nevertheless, most studies were cross-sectional investigations, which could not prove the causal relationship between shift work and hepatic outcomes, and the association between shift work and liver abnormalities has remained inconsistent.[21,22] Thus, this retrospective cohort study investigated the associations of the shift status and the frequency and duration of shift work with the incidence rates of liver enzyme abnormalities (e-ALT and e-AST) and NAFLD as evaluated by LAP and HSI, which can be calculated from existing parameters in our check-up database, among Thai workers in Bangkok, Thailand.

METHODS

Study population

This retrospective cohort study was conducted using the database of workers of the Thai Red Cross Society (a humanitarian agency consisting of a large tertiary hospital and other health and humanitarian

agencies) and Chulalongkorn University (Bangkok, Thailand). The workers in the database provided informed consent for an investigation into the risks of type 2 diabetes mellitus (DM) and abnormal fasting blood glucose (FBG) attributable to shift work and answered self-administered questionnaires in 2016.[23] Job descriptions varied among the workers and included healthcare professionals, academic personnel, security guards, and cleaning staff. Out of 10,205 consenting workers, records of two annual health check-ups conducted from 2009–2016 were available for 6892 workers (67.5%). Of these, 6459 participants with data for weight, height, waist circumference (WC), AST, ALT, and triglyceride (TG) from at least two health

check-ups in 2009–2016 were included in the study. The exclusion criteria were as follows: at least 60 years old (n = 234); a lack of shift work information (n = 71); presence of underlying liver diseases (n = 18); heavy alcohol drinkers (more than 210 g/week for men and more than 140 g/week for women; n = 21);[24] and abnormal values for the outcomes of interest (AST > 35 U/L or ALT > 40 U/L [n = 589]; LAP > 30.5 [men] or LAP > 23 [women]; HSI > 36 [n = 1906]). Accordingly, 3620 workers (2980 women and 640 men) with normal liver enzyme levels and NAFLD diagnostic scores at baseline were followed up until the onset of abnormal values for the outcomes of interest (Figure 1).
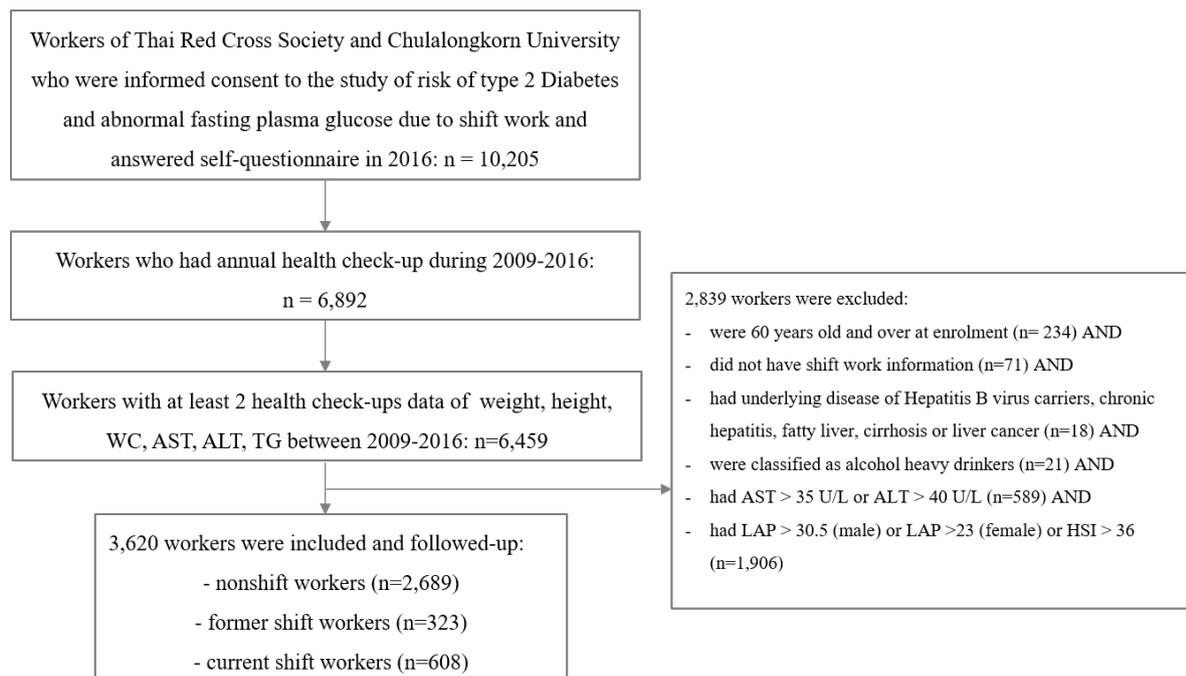
Workers of Thai Red Cross Society and Chulalongkorn University who were informed consent to the study of risk of type 2 Diabetes and abnormal fasting plasma glucose due to shift work and answered self-questionnaire in 2016: n = 10,205

Workers who had annual health check-up during 2009-2016: n = 6,892

Workers with at least 2 health check-ups data of weight, height, WC, AST, ALT, TG between 2009-2016: n=6,459

3,620 workers were included and followed-up:
- nonshift workers (n=2,689)
- former shift workers (n=323)
- current shift workers (n=608)

2,839 workers were excluded:
- were 60 years old and over at enrolment (n= 234) AND
- did not have shift work information (n=71) AND
- had underlying disease of Hepatitis B virus carriers, chronic hepatitis, fatty liver, cirrhosis or liver cancer (n=18) AND
- were classified as alcohol heavy drinkers (n=21) AND
- had AST > 35 U/L or ALT > 40 U/L (n=589) AND
- had LAP > 30.5 (male) or LAP >23 (female) or HSI > 36 (n=1,906)

Figure 1 Flow chart presenting the participant selection with inclusion and exclusion criteria.

Definition of shift work

In this study, the shift work status, frequency (times/month) of shift work, and duration (years) of shift work were the independent variables of interest. The history of shift work among the study participants has been retrospectively assessed by the questionnaire and recorded in the database since 2016. Night shift work

was defined as any work performed for least 3 h consecutively between 00:00 and 06:00.[25] Consequently, the eligible participants were categorized into three groups by their shift work status: (a) "non-shift workers" were participants who had no experience with night shift work or who performed night shift work fewer less than 4 nights/month; (b) "former shift workers"

were participants who had a history of night shift work at least 4 nights/month but were not performing shift work at the start of the study; and (c) "current shift workers" were participants who currently had fixed or rotating night shift work at least 4 nights/month. The frequencies of shift work were categorized as <8 times/month or ≥8 times/month. The duration of shift work exposure was categorized into fewer than 10 years, 10–20 years, or more than 20 years.

Health outcomes

This study had two main aspects of outcomes of interest.

(1) Liver enzyme abnormalities, namely (a) e-ALT (ALT level > 40 U/L) and (b) e-AST (AST level > 35 U/L).

(2) Diagnostic scores for NAFLD

(a) LAP was calculated using the following gender-specific formulas.

Men: (WC in cm − 65) × TG level (mmol/L)

Women: (WC in cm − 58) × TG level (mmol/L)

The cutoff for the presence of NAFLD as indicated by LAP > 30.5 in men and LAP > 23.0 in women.[11]

(b) HSI was calculated using the following formula:

(8 × ALT/AST ratio) + body mass index (BMI, in kg/m^2) + presence of type 2 DM (presence = 2, absence = 0) + female gender (yes = 2, no = 0).

The cutoff for the presence of NAFLD as indicated by HSI > 36.[10]

The sensitivity and specificity of LAP and HSI for the diagnosis of NAFLD were reported in previous studies. The sensitivities of LAP and HSI were 94% and 93.1%, respectively, and their specificities were 85% and 92.4%, respectively.[10,26]

Follow-up and person-years

The eligible participants were followed up until the onset of abnormal outcomes of interests, they reached an age of 60, their data were lost, or the end of the follow-up period was reached (December 2016), whichever came first.

The person-years of follow-up for each participant was calculated from the enrollment year to the year in which abnormal values of the outcomes of interest occurred, participants turned 60, their data were lost, or the end of the follow-up period was reached. The interval between two consecutive health check-ups was 1 year.

Covariate data extraction

The covariates with evidence of roles in the relationship between shift work and the outcomes of interest were extracted from the database. These covariates included demographic variables (gender, age, education level, job type, and marital status), personal health history variables (hypertension, type 2 DM, and dyslipidemia [DLP] as underlying diseases), health behaviors (smoking, alcohol, and exercise habits), and baseline physiologic and biochemical parameters (BMI, WC, systolic blood pressure [SBP], diastolic blood pressure [DBP], AST, ALT, FBG, TG, and high-density lipoprotein cholesterol [HDL-cholesterol]).

Statistical analyses

Descriptive statistics were used to describe the baseline characteristics of participants including demographic, physiological, and biochemical data. Frequencies were presented as numbers, and percentages were calculated for categorical variables. Means and standard deviations were calculated for all normally distributed continuous variables, excluding TG, which had a skewed distribution, and thus, medians with interquartile ranges were calculated. The differences in continuous data among the shift status

groups were analyzed using one-way ANOVA. In the case of significant differences among these groups, Bonferroni's method was performed to identify the different pairs. Fisher's exact test was used to compare the differences between groups of categorical data.

The incidence rates and 95% CIs of e-ALT or e-AST and NAFLD diagnostic scores (e-LAP or e-HSI) were calculated as the number of new cases with abnormal values for the outcomes of interest divided by person-years of follow-up and reported as the incidence rates per 1000 person-years.

The associations of the shift work status, frequency of shift work, and duration of shift work with abnormal outcomes of interest were examined by Cox proportional hazard models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). Potential confounders were managed by adding them into the existing equation for the main exposure (shift work) and outcome (e-ALT, e-AST, e-LAP, or e-HSI) association. To avoid multicollinearity among confounders, the correlation matrix was examined, and one of two factors correlated with each other (correlation coefficient >0.7) was added to the final model. Finally, the estimated HRs were constructed in two models: a crude model and an adjusted model, which was adjusted for confounders, namely age, gender, marital status, education level, job type, alcohol drinking status, BMI at baseline, ALT at baseline, and TG at baseline, history of DLP, exercise status, smoking status, SBP at baseline, FBG at baseline, and HDL-cholesterol at baseline. All Cox regression models were tested for the proportional hazard assumption by "estat phtest" (pass, $p > 0.05$), a test based on Schoenfeld residuals after fitting a model with stcox.[27]

There were missing data for some demographic, physiological, and biochemical variables, as presented in Table 1. These missing data were managed via multiple imputation prior to multivariate HRs analyses.

After an association between the duration of shift work exposure and risk of abnormal NAFLD diagnostic scores was detected, we further investigated the possible pathways that may explain these findings by subgroup risk analysis of e-LAP and e-HSI between nonhealthcare and healthcare shift workers in each group of shift work duration.

The significant level was determined as $p < 0.05$. All statistical analyses were performed using STATA version 17 software (StataCorp, College Station, TX, USA).

Ethics approval

This study was approved by the Institutional Review Board of the Faculty of Medicine of Chulalongkorn University (COA No. 0171/2023).

RESULTS

Participant characteristics

According to the shift work status, 3620 eligible participants were classified into three subgroups, including 2689 non-shift workers, 323 former shift workers, and 608 current shift workers (Figure 1). Most participants in each subgroup were women (80.1%–90.1%). The mean age of current shift workers was 39.4±6.35 years, which was significantly lower than those of non-shift workers (41.2±6.77 years) and former shift workers (42.0±6.69 years). There were significant differences in demographic data among the three subgroups. Whereas 78.1% and 84.5% of non-shift workers and former shift workers, respectively, had bachelor's degrees or higher, only 55.9% of current shift workers reached this level of education. The proportion of participants who were single, widowed, or divorced was higher for current shift workers (52.4%) than for non-shift workers (45.3%) and former shift workers (36.3%). The most common underlying disease at baseline was

DLP, which was less frequent among current shift workers (5.0%) than among non-shift and former shift workers. Concerning health behaviors, whereas most participants in every subgroup (>80%) were non-smokers and non-drinkers, approximately half of them regularly exercised. Regarding the job description, most current shift workers were healthcare professionals (76.6%), and this proportion was higher than those for non-shift workers and former shift workers (Table 1).

Table 1 Baseline characteristics of the eligible participants (n = 3620)

Characteristics	Non-shift workers (n = 2689)		Former shift workers (n = 323)		Current shift workers (n = 608)		One-way ANOVA Welch's F	Post hoc test (Bonferroni)		
	n	%	n	%	n	%		Group comp.	Mean diff.	P-value
Demographic data										
Sex[a]										
Female	2153	80.1	279	86.4	548	90.1				
Age (years), mean (SD) [a]	41.2 (6.77)		42.0 (6.69)		39.4 (6.35)[b]		F(2,3617) = 20.80, p < 0.0001	0 vs. 1	0.85	0.09
								0 vs. 2	**−1.72**	**<0.001**
								1 vs. 2	**−2.57**	**<0.001**
Education level[†,a]										
Primary or illiterate	66	2.5	4	1.2	41	6.8				
Middle or high school	338	12.6	32	9.9	150	24.8				
College or certificate	182	6.8	14	4.4	76	12.5				
Bachelor degree or higher	2095	78.1	272	84.5	339	55.9				
Marital status[†,a]										
Single/widowed/divorced	1217	45.3	117	36.3	318	52.4				
In a relationship/married	1468	54.7	205	63.7	289	47.6				
Underlying diseases at baseline										
HT[†,a]	100	3.9	13	4.3	8	1.4				
Type 2 DM[†]	10	0.4	0	0	1	0.2				
DLP[†,a]	250	11.3	32	12.6	26	5.0				
Smoking status										
Non-smoker	2578	95.9	311	96.3	591	97.2				
Ex-smoker	64	2.4	8	2.5	8	1.3				
Current smoker	47	1.7	4	1.2	9	1.5				
Alcohol drinking status										
Non-drinker	2369	88.1	287	88.9	555	91.3				
Social drinker	320	11.9	36	11.1	53	8.7				
Exercise[†,a]										
Yes	1371	51.0	163	50.5	273	44.9				
Occupation[†,a]										
Healthcare professional	419	15.7	196	60.7	466	76.6				

Characteristics	Non-shift workers (n = 2689)		Former shift workers (n = 323)		Current shift workers (n = 608)		One-way ANOVA Welch's F	Post hoc test (Bonferroni)		
	n	%	n	%	n	%		Group comp.	Mean diff.	P-value
Academic personnel	552	20.7	22	6.8	8	1.3				
Security guards and cleaning staff	40	1.5	5	1.5	41	6.7				
Other roles	1652	62.1	100	31.0	93	15.3				
Baseline physiological and biochemical data, mean (SD)										
BMI (kg/m^2)	21.7 (2.34)		21.4 (2.14)		21.7 (2.34)		$F_{(2,3617)} = 1.31$, $p = 0.27$	0 vs. 1	−0.21	0.39
								0 vs. 2	0.04	1.00
								1 vs. 2	0.24	0.38
WC (cm)										
Women	70.4 (5.99)		70.1 (5.58)		70.7 (5.85)		$F_{(2,2977)} = 0.93$, $p = 0.39$	0 vs. 1	−0.33	1.00
								0 vs. 2	0.25	1.00
								1 vs. 2	0.59	0.54
Men	79.6 (6.34)		78.8 (6.42)		78.0 (5.83)		$F_{(2,637)} = 2.04$, $p = 0.13$	0 vs. 1	−0.83	1.00
								0 vs. 2	−1.63	0.17
								1 vs. 2	−0.81	1.00
SBP (mmHg)[†,a]	115.2 (12.58)		113.7 (12.32)		112.0 (11.72)		$F_{(2,3608)} = 17.25$, $p < 0.0001$	0 vs. 1	−1.48	0.13
								0 vs. 2	**−3.21**	**<0.001**
								1 vs. 2	−1.72	0.13
DBP (mmHg)[†,a]	73.7 (9.17)		72.8 (9.52)		72.1 (8.57)		$F_{(2,3608)} = 8.28$, $p = 0.0003$	0 vs. 1	−0.84	0.35
								0 vs. 2	**−1.61**	**<0.001**
								1 vs. 2	−0.77	0.66
AST (U/L)	18.8 (4.46)		18.5 (4.04)		18.5 (4.17)		$F_{(2,3617)} = 1.12$, $p = 0.33$	0 vs. 1	−0.25	1.00
								0 vs. 2	−0.25	0.61
								1 vs. 2	−0.004	1.00
ALT (U/L)[a]	14.7 (6.35)		14.7 (5.76)		13.9 (5.85)		$F_{(2,3617)} = 3.65$, $p = 0.03$	0 vs. 1	0.03	1.00
								0 vs. 2	**−0.74**	**0.02**
								1 vs. 2	−0.77	0.22
FBG (mg/dL)[†,a]	86.8 (7.91)		86.5 (7.34)		85.6 (7.34)		$F_{(2,3587)} = 6.08$, $p = 0.002$	0 vs. 1	−0.29	1.00
								0 vs. 2	**−1.23**	**0.002**
								1 vs. 2	−0.94	0.25

Characteristics	Non-shift workers (n = 2689)		Former shift workers (n = 323)		Current shift workers (n = 608)		One-way ANOVA Welch's F	Post hoc test (Bonferroni)		
	n	%	n	%	n	%		Group comp.	Mean diff.	P-value
TG (mmol/L), median (IQR)[a]	0.8 (0.38)		0.8 (0.40)		0.8 (0.37)		$F_{(2,3617)} = 6.37$, = 0.002	0 vs. 1	−0.01	1.00
								0 vs. 2	**−0.05**	**0.001**
								1 vs. 2	−0.04	0.24
HDL-cholesterol (mg/dL)[†]										
Women	65.5 (14.31)		66.3 (14.19)		65.0 (14.19)		$F_{(2,2922)} = 0.75$, p = 0.47	0 vs. 1	0.85	1.00
								0 vs. 2	−0.44	1.00
								1 vs. 2	−1.29	0.66
Men	55.3 (12.50)		55.5 (11.25)		52.7 (12.90)		$F_{(2,604)} = 1.21$, p = 0.30	0 vs. 1	0.27	1.00
								0 vs. 2	−2.60	0.38
								1 vs. 2	−2.87	0.75

*Notes: [†]Presence of missing data. [a]Significant difference among shift work status (p < 0.05). Significant difference between group comparison at P-value < 0.05 presented in bold. Group 0: non-shift workers; group 1: former shift workers; group 2: current shift workers. Gray shade: One-way ANOVA could not be applied. **Abbreviations:** Group comp, group comparison; mean diff, mean difference; SD, standard deviation; HT, hypertension; DM, diabetes mellitus; DLP, dyslipidemia; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; FBG, fasting blood sugar; TG, triglyceride; HDL-cholesterol, high-density lipoprotein cholesterol*

In addition to demographic data, physiological and biochemical data at baseline were compared among workers with different shift status. BMI, WC, AST, HDL-cholesterol, LAP, and HSI did not significantly differ among the three subgroups. However, SBP, DBP, ALT, FBG, and TG were significantly lower among current shift workers than among non-shift workers (Table 1).

Incidence rates of NAFLD and liver enzyme abnormalities

Throughout 16,113 (range, 1–7; mean, 4.5) and 16,705 person-years (range, 1–7; mean, 5.3) of NAFLD diagnosed by e-LAP and e-HSI, respectively, the incidence rates of e-LAP and e-HSI were 81.74 and 6.23 per 1000 person-years, respectively. Meanwhile, the incidence rates of e-ALT and e-AST were 19.37 and 17.29 per 1000 person-years, respectively. When the subgroups of shift work were categorized, the incidence rates of all outcomes of interest were higher among non-shift workers than in the other two subgroups (Table 2).

Among shift workers, the incidence rates (per 1000 person-year) of NAFLD, e-ALT, and e-AST in current shift workers who performed night shift work at least 8 times/month were higher than those in their counterparts who performed night shift work fewer than 8 times/month (Table 2). In contrast to the frequency of night shift work, the incidence rates of the outcomes of interest were not increased by the duration of shift work. Current shift workers who had performed shift work for fewer than 10 years had the highest incidence rates of e-ALT, e-AST, and e-HSI, whereas the highest incidence rate of e-LAP was found among participants with 10–20 years of shift work (Table 2).

Table 2 Incidence rates of liver enzyme abnormalities and nonalcoholic fatty liver disease outcomes

Shift work parameters	Outcomes							
	e-LAP		e-HSI		e-ALT		e-AST	
	IR[II]	(95% CI)	IR[II]	(95% CI)	IR[II]	(95% CI)	IR[II]	(95% CI)
Total	81.74	(77.44–86.27)	6.23	(5.14–7.54)	19.37	(17.46–21.49)	17.29	(15.50–19.29)
Shift work status								
Non-shift workers	84.89	(79.79–90.32)	6.52	(5.24–8.13)	20.12	(17.86–22.66)	18.48	(16.33–20.91)
Former shift workers	65.49	(53.83–79.67)	5.21	(2.61–10.42)	18.41	(12.95–26.18)	13.53	(8.99–20.36)
Current shift workers	77.33	(67.70–88.34)	5.49	(3.36–8.96)	16.79	(12.89–21.87)	14.30	(10.75–19.04)
Frequency of shift work								
Non-shift workers	84.89	(79.79–90.32)	6.52	(5.24–8.13)	20.12	(17.86–22.66)	18.48	(16.33–20.91)
Former shift workers	65.49	(53.83–79.67)	5.21	(2.61–10.42)	18.41	(12.95–26.18)	13.53	(8.99–20.36)
Current shift workers								
<8 times/month	73.09	(60.63–88.11)	5.10	(2.55–10.19)	15.40	(10.56–22.46)	13.61	(9.12–20.31)
≥8 times/month	82.24	(68.05–99.40)	5.95	(2.98–11.90)	18.38	(12.69–26.63)	15.10	(10.03–22.73)
Duration of shift work								
Non-shift workers	84.89	(79.79–90.32)	6.52	(5.24–8.13)	20.12	(17.86–22.66)	18.48	(16.33–20.91)
Former shift workers	65.49	(53.83–79.67)	5.21	(2.61–10.42)	18.41	(12.95–26.18)	13.53	(8.99–20.36)
Current shift workers								
<10 years	83.33	(47.33–146.74)	22.39	(7.22–69.42)	46.78	(23.40–93.55)	23.95	(8.99–63.82)
10–20 years	86.38	(71.28–104.68)	3.47	(1.30–9.24)	13.45	(8.36–21.63)	11.92	(7.19–19.78)
>20 years	69.27	(57.00–84.19)	5.54	(2.88–10.64)	16.30	(11.39–23.31)	15.05	(10.39–21.79)

Note: [II]*Incidence rate (IR) per 1000 person-years.* **Abbreviations:** *e-LAP, elevated lipid accumulation product; e-HSI, elevated hepatic steatosis index; e-ALT, elevated alanine aminotransferase; e-AST, elevated aspartate aminotransferase.*

Association between shift work status, NAFLD, and liver enzyme abnormalities

The shift work status was not associated with NAFLD determined by both e-LAP and e-HSI. In former shift workers, the adjusted HR (95% CI) for e-LAP and e-HSI were 0.82 (0.66–1.02) and 0.97 (0.45–2.08), respectively. In current shift workers, the adjusted HR (95% CI) for e-LAP and e-HSI were 0.95 (0.80–1.13) and 0.99 (0.52–1.89), consecutively. All results are presented in Table 3.

Similarly, former and current shift work had no associations with liver enzyme abnormalities (e-ALT and e-AST). The adjusted HR (95% CI) for e-ALT were 1.02 (0.69–1.52) and 0.88 (0.62–1.25) in former and current shift workers, respectively (Table 3).

Likewise, the adjusted HR (95% CI) for e-AST were 0.80 (0.51–1.25) and 0.90 (0.62–1.30) in former and current shift workers, sequentially (Table 3).

Associations of the frequency and duration of shift work exposure with NAFLD and liver enzyme abnormalities

Among shift workers, the frequency of shift work was not associated with any abnormal liver outcomes (Tables 3). Likewise, the duration of shift work had no relationships with liver enzyme abnormalities (Table 3). However, compared to non-shift workers, current shift workers with 10–20 years of experience had a higher risk of e-LAP (adjusted HR [95% CI] = 1.31 [1.04–1.65]), and current shift workers with more than 20 years of experience had a lower risk of e-LAP (adjusted HR [95% CI] = 0.77 [0.62–0.97]; Table 3). Comparably, participants with fewer than 10 years had a higher risk of e-HSI (adjusted HR [95% CI] = 4.30 [1.28–14.47]), but there was no association between the duration of shift work and e-HSI in the other groups (Table 3).

Table 3 Hazard ratios of the associations of shift work parameters with abnormal liver outcomes

Shift work parameters	e-LAP crude HR	e-LAP (95% CI)	e-LAP HR[1]	e-LAP (95% CI)	e-HSI crude HR	e-HSI (95% CI)	e-HSI HR[1]	e-HSI (95% CI)	e-ALT crude HR	e-ALT (95% CI)	e-ALT HR[1]	e-ALT (95% CI)	e-AST crude HR	e-AST (95% CI)	e-AST HR[1]	e-AST (95% CI)
Shift work status																
Non-shift workers		reference				reference				reference				reference		
Former shift workers	0.77	(0.63–0.95)	0.82	(0.66–1.02)	0.79	(0.38–1.63)	0.97	(0.45–2.08)	0.92	(0.63–1.33)	1.02	(0.69–1.52)	0.73	(0.48–1.13)	0.80	(0.51–1.25)
Current shift workers	0.91	(0.78–1.05)	0.95	(0.80–1.13)	0.82	(0.48–1.41)	0.99	(0.52–1.89)	0.84	(0.63–1.12)	0.88	(0.62–1.25)	0.78	(0.57–1.06)	0.90	(0.62–1.30)
Frequency of shift work																
Non-shift workers		reference				reference				reference				reference		
Former shift workers	0.77	(0.63–0.95)	0.82	(0.66–1.02)	0.79	(0.38–1.63)	0.97	(0.45–2.08)	0.92	(0.63–1.33)	1.03	(0.69–1.52)	0.73	(0.48–1.13)	0.80	(0.51–1.25)
Current shift workers																
<8 times/month	0.86	(0.71–1.05)	0.92	(0.74–1.14)	0.76	(0.37–1.58)	0.91	(0.40–2.08)	0.77	(0.52–1.15)	0.96	(0.61–1.49)	0.74	(0.49–1.12)	0.94	(0.59–1.50)
≥8 times/month	0.97	(0.79–1.18)	0.99	(0.79–1.23)	0.89	(0.43–1.84)	1.07	(0.48–2.40)	0.92	(0.62–1.36)	0.82	(0.53–1.26)	0.82	(0.53–1.25)	0.86	(0.53–1.38)
Duration of shift work																
Non-shift workers		reference				reference				reference				reference		
Former shift workers	0.77	(0.63–0.95)	0.83	(0.66–1.03)	0.79	(0.38–1.63)	0.97	(0.45–2.07)	0.92	(0.63–1.33)	1.01	(0.69–1.50)	0.73	(0.48–1.13)	0.80	(0.51–1.24)
Current shift workers																
<10 years	0.99	(0.56–1.75)	0.74	(0.41–1.32)	3.66	(1.15–11.58)	**4.30**	**(1.28–14.47)**	2.27	(1.12–4.58)	2.09	(1.00–4.40)	1.27	(0.47–3.42)	1.57	(0.57–4.33)
10–20 years	1.03	(0.84–1.26)	**1.31**	**(1.04–1.65)**	0.54	(0.20–1.48)	0.63	(0.22–1.87)	0.67	(0.41–1.09)	0.79	(0.46–1.35)	0.64	(0.38–1.08)	0.90	(0.51–1.58)
>20 years	0.80	(0.66–0.99)	**0.77**	**(0.62–0.97)**	0.80	(0.40–1.59)	0.96	(0.44–2.11)	0.82	(0.56–1.20)	0.81	(0.52–1.24)	0.82	(0.55–1.21)	0.84	(0.54–1.32)

Note: 1adjusted HR for age, gender, marital status, education level, job type, alcohol drinking status, body mass index, alanine aminotransferase, and triglycerides at baseline, history of dyslipidemia, exercise status, smoking status, systolic blood pressure, fasting blood sugar, and high-density lipoprotein cholesterol at baseline.
Abbreviations: e-LAP, elevated lipid accumulation product; e-HSI, elevated hepatic steatosis index; e-ALT, elevated alanine aminotransferase; e-AST, elevated aspartate aminotransferase

Subgroup analysis of NAFLD risk with shift work duration

As majority of current shift workers were healthcare personnel, whose workload of shift work decreased as the duration of shift work increased, subgroup analysis for healthcare and non-healthcare shift workers were performed separately. The results showed the significant association between durations of current shift work (10–20 years; >20 years) and e-LAP in healthcare shift workers (Figure 2). Conversely, the significant association between duration of <10 years of shift work and e-HSI was observed in non-healthcare shift workers (Figure 3).
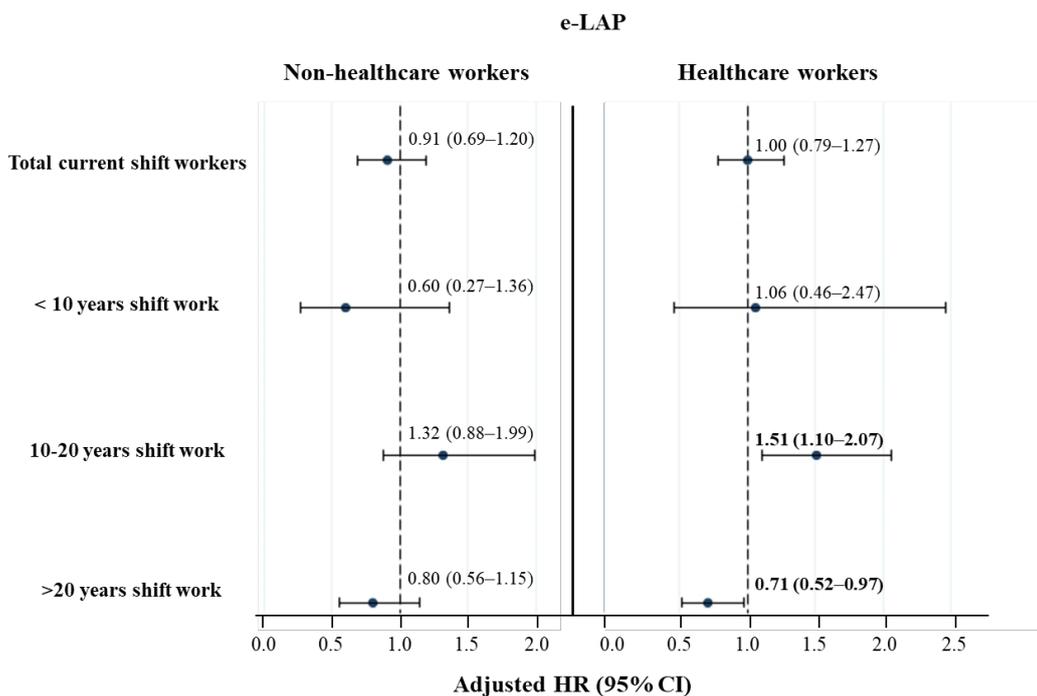
Figure 2 Subgroup analysis of the associations of shift work duration with e-LAP
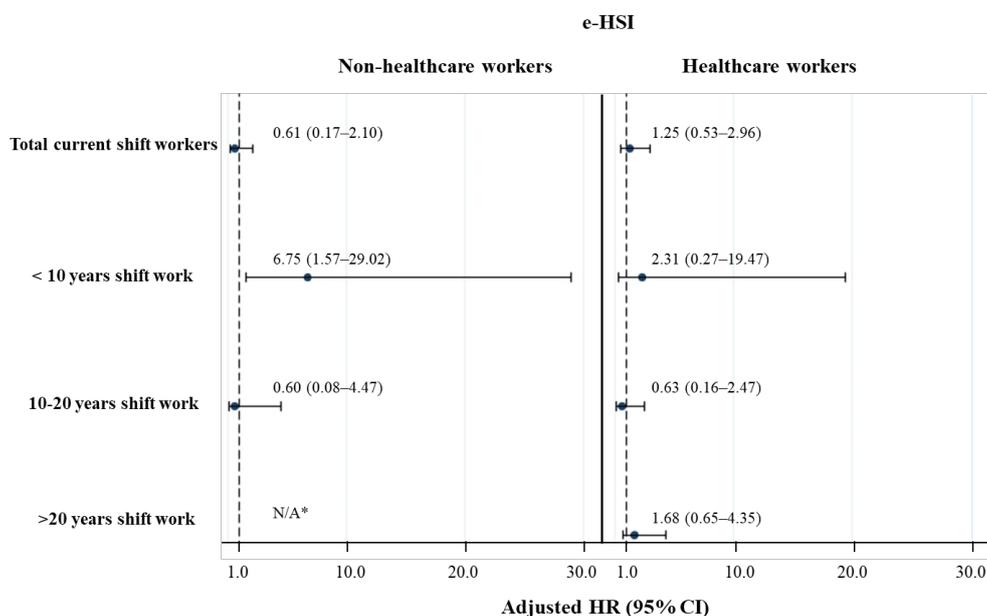
Figure 3 Subgroup analysis of the associations of shift work duration with e-HSI

Note *no available data in this subgroup

DISCUSSION

Main findings

Our results illustrated that the overall incidence of NAFLD, as evaluated by LAP, was higher than the incidence of liver enzyme abnormalities. In addition, our cohort study demonstrated that the status, frequency, and duration of shift work were not associated with liver enzyme abnormalities. Although the status and frequency of shift work were not related to the incidence of NAFLD, 10–20 years and fewer than 10 years of shift work experience increased the risk of NAFLD as assessed by e-LAP in healthcare shift workers and e-HSI in non-healthcare shift workers, respectively, without a dose–response relationship pattern. Moreover, healthcare personnel with more than 20 years of shift work experience had a lower risk of e-LAP.

The differences in liver abnormalities

Because of the spectrum of NAFLD, lipid accumulation in liver cells can occur without causing liver cell injury.[28] This phenomenon could explain why the incidence of NAFLD was higher than that of liver enzyme elevation, which is indicative of liver cell injury, and our results confirmed that liver enzyme elevation is not sensitive for NAFLD detection.[29] In addition, the NAFLD diagnostic scores evaluated in this study (LAP and HSI) were inconsistent. Although the accuracy of these two scores has been proven in the Asian population, the accuracy of LAP was better in younger participants (18–34 years for men; 18–34 and 35–44 years for women)[10,11] and in those with hyperlipidemia,[30] which was a common underlying disease in this study.

The associations of shift work with NAFLD and liver enzyme abnormalities

Our recent findings were consistent with two previous cross-sectional studies that found no association of shift work with e-ALT and e-AST.[21,22] The shared characteristics between these two studies and our study were the variety of jobs among shift workers. However, the association between shift work and NAFLD, as assessed by either imaging or NAFLD diagnostic scores, was not evaluated in the prior studies. In contrast to the results of a 4-year longitudinal cohort study among Chinese rail workers,[31] our results revealed no association of the status and frequency of shift work with NAFLD. This discrepancy in findings is suggested to arise from differences in job exposure, population characteristics, and NAFLD assessment. In detail, the participants in the study by Xu *et al.* were mainly men, who have a higher prevalence of NAFLD.[32] Moreover, although the shift workers in their study were confined to the rail business,[31] there was heterogeneity in the job description among the shift workers in our study.

Previous studies on the association between the duration of shift work and NAFLD outcomes reported inconsistent findings.[19,33,34] Conversely, our findings revealed that shift work durations of 10–20 years and fewer than 10 years were risk factors for NAFLD in healthcare and non-healthcare shift workers, consecutively, but a long duration (>20 years) of shift work was specifically a protective factor for e-LAP in healthcare personnel. The differences between our results and those of previous studies could be explained by the following possible explanations: the workload of shift workers in Thailand, particularly healthcare personnel, decreased as the duration of shift work increased; or our biological clocks can be adapted to the shift work schedules as time passes.[35] Moreover, the "healthy worker effect" could be an alternative explanation for why current shift workers with a long duration of shift work who remain healthy

have a decreased risk of NAFLD, an indicator of metabolic dysfunction.[28,36]

Strengths and limitation of the study

The major strength of our study included the use of multiple NAFLD diagnostic scores and a simultaneous evaluation of e-ALT and e-AST in a retrospective cohort study. In addition to the shift status, the frequency and duration of shift work were independent variables of interest. However, this study had several limitations, including the lack of testing to compare the performance of LAP and HSI to liver biopsy (gold standard for NAFLD diagnosis), which could have resulted in nondifferential outcome misclassification and bias toward the null of the HR estimate.[37] Other limitations included heterogeneity regarding the job description among shift workers, which could affect the workload, and the female predominance of the study population, which could limit the generalizability of the findings to the general population.

RECOMMENDATIONS

While the overall association between the shift work status and NAFLD was not observed, we found inconsistent findings between the association of shift work duration and NAFLD. Further longitudinal studies with sufficiently large sample sizes incorporating more detailed information on shift work characteristics and thorough potential confounding factors are therefore suggested to clearly elucidate these issues. Notwithstanding these inconclusive results, regular workplace NFALD screening program using simple and inexpensive diagnostic scores such as LAP and HSI should be implemented, particularly among shift workers in their early 20 work years, to avoid serious long-term consequence of NAFLD incurring upon the workers.

REFERENCES

1. Allen AM, Lazarus JV, Younossi ZM. Healthcare and socioeconomic costs of NAFLD: A global framework to navigate the uncertainties. J Hepatol. 2023;79(1):209-17. doi: 10.1016/j.jhep.2023.01.026.
2. Cotter TG, Rinella M. Nonalcoholic Fatty Liver Disease 2020: The State of the Disease. Gastroenterology. 2020;158(7):1851-64. doi: 10.1053/j.gastro.2020.01.052.
3. Shah PA, Patil R, Harrison SA. NAFLD-related hepatocellular carcinoma: The growing challenge. Hepatology. 2023;77(1):323-38. doi: 10.1002/hep.32542.
4. Younossi ZM. Non-alcoholic fatty liver disease - A global public health perspective. J Hepatol. 2019;70(3):531-44. doi: 10.1016/j.jhep.2018.10.033.
5. Witkowski M, Moreno SI, Fernandes J, Johansen P, Augusto M, Nair S. The Economic Burden of Non-Alcoholic Steatohepatitis: A Systematic Review. Pharmacoeconomics. 2022;40(8):751-76. doi: 10.1007/s40273-022-01140-y.
6. National Institute for Health and Care Excellence (NICE). Non-alcoholic fatty liver disease (NAFLD): assessment and management, 2016 [Internet]. [cited 2023 Nov 1]. Available from:

https://www.nice.org.uk/guidance/ng4 9.

7. Bedogni G, Kahn HS, Bellentani S, Tiribelli C. A simple index of lipid overaccumulation is a good marker of liver steatosis. BMC Gastroenterol. 2010;10:98. doi: 10.1186/1471-230X-10-98.

8. Kahn HS. The "lipid accumulation product" performs better than the body mass index for recognizing cardiovascular risk: a population-based comparison. BMC Cardiovasc Disord. 2005;5:26. doi: 10.1186/1471-2261-5-26.

9. Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, et al. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology. 2009;137(3):865-72. doi: 10.1053/j.gastro.2009.06.005.

10. Lee JH, Kim D, Kim HJ, Lee CH, Yang JI, Kim W, et al. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. Dig Liver Dis. 2010;42(7):503-8. doi: 10.1016/j.dld.2009.08.002.

11. Dai H, Wang W, Chen R, Chen Z, Lu Y, Yuan H. Lipid accumulation product is a powerful tool to predict non-alcoholic fatty liver disease in Chinese adults. Nutr Metab (Lond). 2017;14:49. doi: 10.1186/s12986-017-0206-2.

12. Reinke H, Asher G. Circadian Clock Control of Liver Metabolic Functions. Gastroenterology. 2016;150(3):574-80. doi: 10.1053/j.gastro.2015.11.043.

13. McCommis KS, Butler AA. The Importance of Keeping Time in the Liver. Endocrinology. 2021;162(2). doi: 10.1210/endocr/bqaa230.

14. Mukherji A, Bailey SM, Staels B, Baumert TF. The circadian clock and liver function in health and disease. J Hepatol. 2019;71(1):200-11. doi: 10.1016/j.jhep.2019.03.020.

15. International Labour Organization. Yearly statistics 2015 [Internet]. [cited 2022 Sept 4]. Available from: http://laborsta.ilo.org.

16. Ministry of Labour. Labour statistics yearbook 2022: The office of permanent secretary, ministry of labour; 2022 [Internet]. [cited 2023 Nov 1]. Available from: https://www.mol.go.th/academician/reportstatic_labour.

17. Lee S, Messenger JC. Working time around the world: trends in working hours, laws and policies, laws and policies in a global comparative perspective. Routledge: Milton Park; 2007.

18. Lin YC, Chen PC. Persistent rotating shift work exposure is a tough second hit contributing to abnormal liver function among on-site workers having sonographic fatty liver. Asia Pac J Public Health. 2015;27(2):Np1765-74. doi: 10.1177/1010539512469248.

19. Wang F, Zhang L, Wu S, Li W, Sun M, Feng W, et al. Night shift work and abnormal liver function: is non-alcohol fatty liver a necessary mediator? Occup Environ Med. 2019;76(2):83-9. doi: 10.1136/oemed-2018-105273.

20. Zhang P, Wang CY, Li YX, Pan Y, Niu JQ, He SM. Determination of the upper cut-off values of serum alanine aminotransferase and aspartate aminotransferase in Chinese. World J Gastroenterol. 2015;21(8):2419-24. doi: 10.3748/wjg.v21.i8.2419.

21. Balakrishnan M, El-Serag HB, Kanwal F, Thrift AP. Shiftwork Is Not Associated with Increased Risk of NAFLD: Findings from the National Health and Nutrition Examination Survey. Dig Dis Sci. 2017;62(2):526-33. doi: 10.1007/s10620-016-4401-1.

22. Choi H, Oh HJ, Shin JS, Lim M, Kim SK, Kang HT, et al. Relationship between shift work and liver enzymes: a cross-sectional study based on the Korea National Health and

Examination Survey (2007-2015). Ann Occup Environ Med. 2019;31:e15. doi: 10.35371/aoem.2019.31.e15.

23. Hanprathet N, Lertmaharit S, Lohsoonthorn V, Rattananupong T, Ammaranond P, Jiamjarasrangsi W. Increased Risk Of Type 2 Diabetes And Abnormal FPG Due To Shift Work Differs According To Gender: A Retrospective Cohort Study Among Thai Workers In Bangkok, Thailand. Diabetes Metab Syndr Obes. 2019;12:2341-54. doi: 10.2147/DMSO.S219524.

24. Kwo PY, Cohen SM, Lim JK. ACG Clinical Guideline: Evaluation of Abnormal Liver Chemistries. Am J Gastroenterol. 2017;112(1):18-35. doi: 10.1038/ajg.2016.517.

25. The International Agency for Research on Cancer (IARC). Painting, firefighting, and shiftwork: IARC monographs on the evaluation of carcinogenic risks to humans; 2010 [Internet]. [cited 2023 Nov 1]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/21381544.

26. Ebrahimi M, Seyedi SA, Nabipoorashrafi SA, Rabizadeh S, Sarzaeim M, Yadegar A, et al. Lipid accumulation product (LAP) index for the diagnosis of nonalcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis. Lipids Health Dis. 2023;22(1):41. doi: 10.1186/s12944-023-01802-6.

27. Stata. stcox PH-assumption tests-Tests of proportional-hazards assumption after stcox2022 [Internet]. [cited 2023 June 1]. Available from: https://www.stata.com/manuals/ststcoxph-assumptiontests.pdf.

28. Powell EE, Wong VW, Rinella M. Non-alcoholic fatty liver disease. Lancet. 2021;397(10290):2212-24. doi: 10.1016/S0140-6736(20)32511-3.

29. European Association for the Study of the Liver, European Association for the Study of Diabetes, European Association for the Study of Obesity. EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. J Hepatol. 2016;64(6):1388–402. doi: 10.1007/s00125-016-3902-y.

30. Lind L, Johansson L, Ahlström H, Eriksson JW, Larsson A, Risérus U, et al. Comparison of four non-alcoholic fatty liver disease detection scores in a Caucasian population. World J Hepatol. 2020;12(4):149-59. doi: 10.4254/wjh.v12.i4.149.

31. Xu J, Ni S, Wang Y, Yan M, Yang X, Ge H, et al. Shift work and nonalcoholic fatty liver disease incidence among Chinese rail workers: a 4-year longitudinal cohort study. Int Arch Occup Environ Health. 2023;96(1):179-90. doi: 10.1007/s00420-022-01913-0.

32. Burra P, Bizzaro D, Gonta A, Shalaby S, Gambato M, Morelli MC, et al. Clinical impact of sexual dimorphism in non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). Liver Int. 2021;41(8):1713-33. doi: 10.1111/liv.14943.

33. Kim K, Lee YJ, Kwon SC, Min YS, Lee HK, Baek G, et al. Correlation between shift work and non-alcoholic fatty liver disease among male workers in the steel manufacturing company of Korea: a cross-sectional study. Ann Occup Environ Med. 2022;34:e33. doi: 10.35371/aoem.2022.34.e33.

34. Zhang S, Wang Y, Wang Z, Wang H, Xue C, Li Q, et al. Rotating night shift work and non-alcoholic fatty liver disease among steelworkers in China: a cross-sectional survey. Occup Environ Med. 2020;77(5):333-9. doi: 10.1136/oemed-2019-106220.

35. Arendt J. Shift work: coping with the biological clock. Occup Med (Lond). 2010;60(1):10-20. doi: 10.1093/occmed/kqp162.

36. Chowdhury R, Shah D, Payal AR. Healthy Worker Effect Phenomenon: Revisited with Emphasis on Statistical Methods - A Review. Indian J Occup Environ Med. 2017;21(1):2-8. doi: 10.4103/ijoem.IJOEM_53_16.

37. Rothman Kj GS, Lash TL. Modern epidemiology. 3rd ed. PA: Lippincott Williams & Wilkins; 2008.