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Leptospirosis and Peripheral Artery Occlusive Disease

A Nationwide Cohort Analysis

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Abstract: Data on the association between peripheral artery occlusive disease (PAOD) and leptospirosis are limited. We conducted a retrospective cohort study for determining whether leptospirosis is one of the possible risk factors for PAOD.

Patients diagnosed with leptospirosis by using 2000 to 2010 data from the Taiwan National Health Insurance Research Database. Patients with leptospirosis without a history of PAOD were selected. For each leptospirosis patient, 4 controls without a history of leptospirosis and PAOD were randomly selected and frequency-matched for sex, age, the year of the index date, and comorbidity diseases. The follow-up period was from the time of the initial diagnosis of leptospirosis to the diagnosis date of PAOD, or December 31, 2011. The Cox proportional hazard regression models were used for analyzing the risk of PAOD.

During the follow-up period, the cumulative incidence of PAOD was higher among the patients from the leptospirosis cohort than among the nonleptospirosis cohort (log-rank test, $P < 0.001$). In total, 29 patients with PAOD from the leptospirosis cohort and 81 from the nonleptospirosis cohort were observed with the incidence rates of 2.1 and 1.3 per 1000 person-years, respectively, yielding a crude hazards ratio (HR) of 1.62 (95% confidence interval [CI] = 1.44–1.81) and adjusted HR (aHR) of 1.75 (95% CI = 1.58–1.95).

The risk of PAOD was 1.75-fold higher in the patients with leptospirosis than in the general population.

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Abbreviations: aHR = adjusted hazard ratio, BNHI = Bureau of National Health Insurance, CI = confidence interval, LHID 2000 = Longitudinal Health Insurance Database 2000, NHI = National Health Insurance, NHIA = National Health Insurance Administration, NHIRD = National Health Insurance Research Database, NHRI = National Health Research Institutes, PAOD = peripheral artery occlusive disease.

INTRODUCTION

Leptospirosis is one of the most common zoonotic infections worldwide[1] and is usually encountered in the tropics and developing regions;[2–4] however, it has been reported in the European countries and USA.[5–7] It is caused by *Leptospira* spp., which are gram-negative spirochetes comprising 24 serogroups and 250 serovars.[1] Pathogenic *leptospira* colonize the renal tubules of the reservoir hosts and are excreted through urine into the environment. People are infected through contaminated soil and water. The presentation of leptospirosis include asymptomatic illness and mild flu-like symptoms as well as severe disease forms, such as Weil's disease (the triad of jaundice, acute renal failure, and bleeding), chronic interstitial nephritis, mastitis, myocarditis, hemolytic crisis, and multiorgan failure.[8]

Peripheral arterial occlusive disease (PAOD) results from an atherosclerotic process causing stenosis and occlusion of noncerebral and noncoronary arteries. It affects 15% to 20% of people >70 years worldwide.[9–11] The classic symptoms are leg pain while walking, which resolves with rest and intermittent claudication. Complications may include an infection or tissue death, which may require amputation, or even death. The major risk factors for PAOD have been determined from large epidemiologic studies and are consistent with the risk factors for cerebrovascular and ischemic heart diseases. Studies have confirmed that the most crucial risk factors, such as diabetes, hypertension, smoking, and hyperlipidemia, are associated with 80% to 90% of cardiovascular diseases.[12,13]

Studies on the association between PAOD and infection are limited. Because PAOD and cardiovascular diseases share risk factors and because a previous study revealed that leptospirosis may associate to acute coronary syndrome,[14] leptospirosis

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can also be one of the risk factors for PAOD. Thus, we conducted a retrospective cohort study for determining whether leptospirosis is one of the possible risk factors for PAOD.

METHODS

Data Source

In this study, we selected patients from Taiwan's National Health Insurance Research Database (NHIRD), which is managed by the National Health Research Institutes (NHRI). The data in the NHIRD are derived from the National Health Insurance (NHI) program, which was implemented in 1995 and covers >99% of the Taiwan population.[15] The NHIRD details have been adequately described in previous highly cited papers.[16,17] To ensure patient privacy, patient identification numbers are scrambled before the files are released for research. Diseases were defined according to the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes. This study was approved by the Institutional Review Board of China Medical University (CMUH104-REC2-115).

Sampled Patients

We identified patients with the first hospitalization for leptospirosis (ICD-9 code 100) from the inpatient claims data between 2000 and 2010. The date of the first hospitalization for leptospirosis was identified as the index date. We extracted 3458 patients with leptospirosis with complete age and sex information and without a history of PAOD (ICD-9-CM 440.2, 440.3, 443, 444.22, 444.81) before the index date; these patients comprised the leptospirosis cohort. For each patient with leptospirosis, 4 controls with complete age and sex information and without history of leptospirosis and PAOD were randomly selected and were frequency-matched for sex, age (every 5-year span), the year of the index date, and comorbidities of diabetes, hypertension, hyperlipidemia, obesity, chronic obstructive pulmonary disease (COPD), heart failure, coronary artery disease (CAD), stroke, and asthma. In total, 3458 and 13,794 patients with leptospirosis and controls without leptospirosis history, respectively, were followed up until the diagnosis of PAOD, loss to follow-up, death, withdrawal of insurance, and the end of 2011, whichever occurred first.

Comorbidity

Comorbidities, namely diabetes (ICD-9-CM code 250), hypertension (401–405), hyperlipidemia (272), obesity (278), COPD (491, 492, and 496), heart failure (428), CAD (410–414), stroke (430–438), and asthma (493), were analyzed.

Statistical Analysis

We described and compared the distributions of sex, age, and comorbidities (%) between the 2 cohorts by using the chi-square test. Student's *t* test was used for examining the differences in the mean age and follow-up period of the 2 cohorts. The cumulative incidence rate of PAOD was plotted using the Kaplan–Meier method, and the differences between the 2 cohorts were examined using the log-rank test. The incidence density rate (per 1000 person-y) was estimated for both cohorts after stratifying by sex, age, and comorbidities. The uni- and multivariable Cox proportional hazards regression models were used for examining the influence of leptospirosis on the risk of PAOD based on the calculated hazard ratios (HRs) with 95% confidence intervals (CIs). The multivariable model was simultaneously adjusted for age and comorbidities of diabetes, hypertension, hyperlipidemia, COPD, heart failure, CAD, stroke, and asthma. All data processing and statistical analyses were performed using the SAS Version 9.4 (SAS Institute Inc., Cary, NC). All *P* were 2-tailed, and $P < 0.05$ was considered statistically significant.

RESULTS

Demographic Characteristics, Comorbidities, and Cumulative Incidence of PAOD in Leptospirosis Patients and Control Group.

The patients in the leptospirosis and nonleptospirosis cohorts were frequency-matched for sex, age, and comorbidities. Men constituted the majority (68.0% vs 32.0%), and 45.0% of the patients were < 50 years (Table 1). The mean age of the leptospirosis cohort was 52.5 ± 16.4 years and that of the nonleptospirosis cohort was 52.3 ± 16.6 years.

During the mean follow-up periods of 4.00 and 4.52 years for the leptospirosis and nonleptospirosis cohorts, respectively, the cumulative incidence rate of PAOD was higher in the leptospirosis cohort than in the nonleptospirosis cohort (log-rank test, $P < 0.001$) (Figure 1).

Incidence and Hazard ratio of PAOD in Leptospirosis Patients and Control Group and Stratified by Sex, Age, and Comorbidity

In total, 29 patients with PAOD from the leptospirosis and 81 patients from the nonleptospirosis cohort were followed; the incidence rates were 2.10 and 1.30 per 1000 person-years, respectively, yielding a crude HR of 1.62 (95% CI = 1.44–

TABLE 1. Demographic Characteristics and Comorbidity in Patient With and Without Leptospirosis

	Leptospirosis		
	No	Yes	
Variable	N = 13794	N = 3458	*P* Value
Sex	n (%)	n (%)	0.95
Female	4404 (31.9)	1106 (32.0)	
Male	9390 (68.1)	2352 (68.0)	
Age, mean (SD)	52.3 (16.6)*	52.5 (16.4)	0.50
Stratify age			0.99
≤50	1557 (45.0)	1557 (45.0)	
50–65	4088 (29.6)	1025 (29.6)	
65+	3498 (25.4)	876 (25.3)	
Comorbidity			
Diabetes	2044 (14.8)	518 (15.0)	0.81
Hypertension	3001 (21.8)	757 (21.9)	0.86
Hyperlipidemia	867 (6.29)	219 (6.33)	0.92
Obesity	74 (0.54)	25 (0.72)	0.19
COPD	607 (4.40)	155 (4.48)	0.83
Heart failure	679 (4.92)	175 (5.06)	0.74
CAD	1061 (7.69)	269 (7.78)	0.86
Stroke	1117 (8.10)	283 (8.18)	0.87
Asthma	490 (3.55)	130 (3.76)	0.56

* Student's t test.
Chi-Square Test.
CAD = coronary artery disease, COPD = chronic obstructive pulmonary disease, SD = standard deviation.

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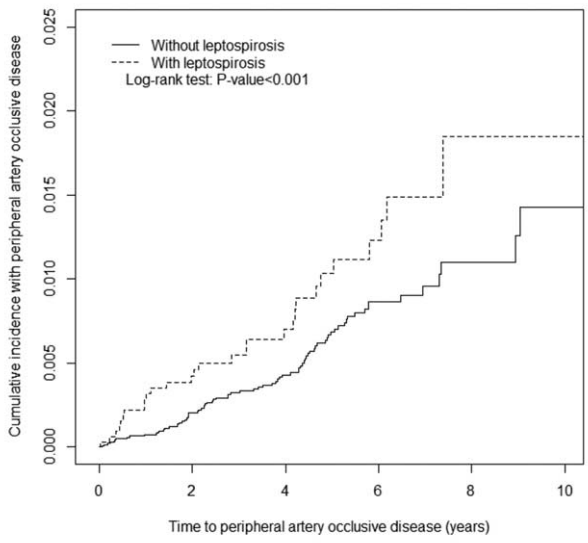

FIGURE 1. Differences in the cummulative incidence rate of peripheral artery occlusive disease between the cohorts with and without leptospirosis by using the Kaplan–Meier method.

1.81) and adjusted HR (aHR) of 1.75 (95% CI = 1.58–1.95) (Table 2). After stratifying by sex, the relative risk of PAOD was higher for both women (aHR = 2.86, 95% CI = 2.41–3.40) and men (aHR = 1.33, 95% CI = 1.16–1.52) in the leptospirosis cohort than for those in the nonleptospirosis cohort. The incidence rate of PAOD increased with age in both cohorts; however, the risk was higher for the patients in the leptospirosis cohort than in the nonleptospirosis cohort despite the age differences. After adjustment for comorbidities, the risk of PAOD was higher for the patients in the leptospirosis cohort than for those in the nonleptospirosis cohort without comorbidities (aHR = 3.09, 95% CI = 2.67–3.56) and with comorbidities (aHR = 1.50, 95% CI = 1.26–1.78).

Incidence and Hazard Ratio of Other Risk Factors

The results of the uni- and multivariable Cox proportional hazards regression models for analyzing the risk factors for PAOD are listed in Table 3. The aHR of PAOD increased by 1.05-fold (95% CI = 1.04–1.05) with age (every year). The risk of PAOD was higher in the patients with comorbidities, namely diabetes (aHR = 4.44, 95% CI = 4.02–4.89), hyperlipidemia (aHR = 1.60, 95% CI = 1.41–1.83), COPD (aHR = 1.38, 95% CI = 1.18–1.60), heart failure (aHR = 2.37, 95% CI = 2.08–2.71), CAD (aHR = 1.49, 95% CI = 1.32–1.68), and stroke (aHR = 1.79, 95% CI = 1.60–2.01).

DISCUSSION

Leptospirosis is one of the most common zoonotic infections worldwide. A study revealed that leptospirosis maybe an independent risk factor for acute coronary syndrome;[14] however, available studies suggesting an association between PAOD and leptospirosis are scarce. This is the first study revealing a 1.75-fold higher risk of PAOD in the patients with leptospirosis than in the general population.

Role of Endothelial Dysfunction and Inflammatory Process in Atherosclerosis and PAOD

PAOD is one of the most common manifestations of atherosclerosis. Smoking, hypertension, diabetes mellitus,

TABLE 2. Comparison of Incidence and Hazard Ratio of Peripheral Artery Occlusive Disease Stratified by Sex, Age, and Comorbidity Between With and Without Leptospirosis Patients

	Without Leptospirosis					With Leptospirosis				
Variables	**Event**	**PY**	**Rate**[†]	**Crude HR**[‡] **(95% CI)**	**Adjusted HR**[§] **(95% CI)**	**Event**	**PY**	**Rate**[†]	**Crude HR**[‡] **(95% CI)**	**Adjusted HR**[§] **(95% CI)**
All	81	62,400	1.30	1 (Reference)	1 (Reference)	29	13,828	2.10	1.62 (1.44, 1.81)**	1.75 (1.58, 1.95)**
Sex										
Female	23	19,707	1.17	1 (Reference)	1 (Reference)	13	4399	2.96	2.53 (2.10, 3.06)**	2.86 (2.41, 3.40)**
Male	58	42,693	1.36	1 (Reference)	1 (Reference)	16	9429	1.70	1.25 (1.08, 1.45)*	1.33 (1.16, 1.52)**
Stratify age										
≤65	37	47,746	0.77	1 (Reference)	1 (Reference)	11	10,909	1.01	1.30 (1.12, 1.51)**	1.34 (1.17,1 .54)**
65+	44	14,653	3.00	1 (Reference)	1 (Reference)	18	2919	6.17	2.05 (1.68, 2.51)**	2.12 (1.74, 2.57)**
Comorbidity[‖]										
No	10	39,953	0.25	1 (Reference)	1 (Reference)	7	9088	0.77	3.08 (2.67, 3.55)**	3.09 (2.67, 3.56)**
Yes	71	22,447	3.16	1 (Reference)	1 (Reference)	22	4740	4.64	1.47 (1.23, 1.75)**	1.50 (1.26, 1.78)**

[†] Rate, incidence rate, per 1,000 person-years.
[‡] Crude HR, crude hazard ratio.
[§] Adjusted HR: multivariable analysis including age, and comorbidities of diabetes, hypertension, hyperlipidemia, COPD, heart failure, CAD, stroke, and asthma.
[‖] Comorbidity: Patients with any one of the comorbidities diabetes, hypertension, hyperlipidemia, obesity, COPD, heart failure, CAD, stroke, and asthma were classified as the comorbidity group.
* $P < 0.01$.
** $P < 0.001$.

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TABLE 3. HR of Peripheral Artery Occlusive Disease in Association With Sex, Age, and Comorbidities in Univariable and Multivariable Cox Regression Models

	Crude†	Adjusted‡
Variables	**HR (95% CI)**	**HR (95% CI)**
Leptospirosis	1.62 (1.44, 1.81)*	1.75 (1.58, 1.95)*
Sex (women vs men)	0.95 (0.85, 1.06)	–
Age, y	1.06 (1.06, 1.07)*	1.05 (1.04, 1.05)*
Baseline comorbidities (yes vs no)		
Diabetes	7.30 (6.62, 8.04)*	4.44 (4.02, 4.89)*
Hypertension	3.28 (2.97, 3.63)*	1.06 (0.95, 1.19)
Hyperlipidemia	3.36 (2.94, 3.84)*	1.60 (1.41, 1.83)*
Obesity	–	–
COPD	4.57 (3.96, 5.27)*	1.38 (1.18, 1.60)*
Heart failure	7.44 (6.61, 8.37)*	2.37 (2.08, 2.71)*
CAD	5.34 (4.79, 5.97)*	1.49 (1.32, 1.68)*
Stroke	4.61 (4.12, 5.17)*	1.79 (1.60, 2.01)*
Asthma	3.74 (3.18, 4.40)*	1.17 (0.99, 1.39)

CAD = coronary artery disease, CI = confidence interval, COPD = chronic obstructive pulmonary disease.

† Crude HR, relative hazard ratio.

‡ Adjusted HR: multivariable analysis including age, and comorbidities of diabetes, hypertension, hyperlipidemia, COPD, heart failure, CAD, stroke, and asthma.

* $P < 0.001$.

and hypercholesterolemia are the well-established key factors for the initiation and development of atherosclerosis and its clinical manifestations.[18,19] Atherosclerosis was initially considered as a cholesterol disease until Ross revealed that it is also an inflammatory disease in 1999.[20] The combined effects of endothelial dysfunction and inflammatory process are considered as the key factors for atherosclerosis and PAOD.[20–22]

The mediators involved in the inflammatory processes leading to PAOD also contribute toward CAD development.[23–28] Atherosclerosis results from an excessive inflammatory and fibroproliferative response to endothelial damage, which alters the normal homeostatic properties of the endothelium,[20,29] subsequently developing processes such as increased endothelial permeability to lipoproteins, upregulation of leukocyte and endothelial adhesion molecules, and migration of leukocytes into the artery wall. Then, the inflammatory process mediated by numerous vasoactive molecules and cytokines stimulates migration and proliferation of smooth muscle cells, which participate in the formation of fatty streaks, characteristic of atherosclerosis, along with lipid-laden monocytes, macrophages, and T lymphocytes.[20] Atherosclerosis plaques are then formed, resulting in PAOD symptoms.

Pre-Existing Factors of Inflammation Leading to Atherosclerosis and PAOD

The various causes of inflammation include cigarette smoking and diabetes mellitus, which promote oxidative stress and induce inflammatory pathways directly or indirectly.[30–33] Furthermore, hypertension induces inflammatory processes, where angiotensin II elicits the production of reactive oxygen species, expression of vascular cell adhesion molecule-1 in the endothelial cells,[34,35] and expression of proinflammatory cytokines, such as interleukin-6 and monocyte chemoattractant protein-1, in the arterial smooth muscle cells,[36] consequently developing atherosclerosis and PAOD. Moreover, infectious agents are possible causes of inflammation leading to atherosclerosis and PAOD. Bloemenkamp et al[37] measured IgG antibody titers in 228 young women with PAOD and 643 control women in a case–control study. Their results showed that the odds ratios for PAOD in women with serological evidence of infections with *Chlamydia pneumonia*, *Helicobacter pylori*, and cytomegalovirus were 2.0 (95% CI = 1.3–3.1), 1.6 (95% CI = 1.1–2.2), and 1.6 (95% CI = 1.1–2.3), respectively. However, the mechanisms underlying these infection-induced developments of atherosclerosis and PAOD remain unknown.

Role of Endothelial Dysfunction and Inflammatory Process in Leptospirosis

Moreover, endothelial damage and systemic inflammation are also the characteristic pathogeneses of leptospirosis. *Leptospira* frequently enter the body via skin abrasion or exposed mucous membranes and spread through the bloodstream and tissues without eliciting initial inflammation. The acute or septicemic phase lasts for ~1 week without considerable complications, followed by the immune phase, characterized by antibody production and excretion of *Leptospira* in the urine. During the immune phase, vasculitis, endothelial damage, and inflammatory infiltrates comprising monocytes, plasma cells, histiocytes, and neutrophils develop in any affected tissue.[19] Endothelial damage and systemic inflammatory processes caused by leptospirosis may result in atherosclerosis, which possibly leads to the development of PAOD and acute coronary syndrome. This could be the possible underlying mechanism in patients with leptospirosis with an increased risk of developing PAOD.

Other Factors Which May Associate to PAOD

In this study, we discovered that the incidence rate of PAOD increased with age in the leptospirosis cohort. The aHR of PAOD increased by 1.05-fold (95% CI = 1.04–1.05) with age (every year). These results are consistent with those of previous reports.[38,39] Furthermore, the risk of PAOD was higher in the patients with comorbidities of diabetes (aHR = 4.44), hypertension (aHR = 1.06), hyperlipidemia (aHR = 1.60), COPD (aHR = 1.38), heart failure (aHR = 2.37), CAD (aHR = 1.49), and stroke (aHR = 1.79). This result indicated that all the aforementioned chronic diseases attributed to at least a part of the pathogenesis of PAOD, such as systemic inflammation and endothelial damage.

Study Limitation

Our study included a large sample of patients because registering to the NHI program is mandatory in Taiwan. The NHI beneficiaries are assigned unique personal identification numbers that enabled us to trace the patients throughout the study follow-up period. However, our study has several limitations. First, the diagnosis of leptospirosis was determined using the ICD-9-CM codes obtained from the NHRID, and leptospirosis presentation with mild symptoms may have been classified by the codes for fever, acute upper respiratory tract infection, or other diseases, thus, underestimating the risk of PAOD. Second, the patients with leptospirosis selected for our study were hospitalized and might have had presented with

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relatively severe symptoms; therefore, the increased risk of PAOD may have only been found in such patients. Third, information on the daily physical activity, diet, patient behavior, such as smoking and exercise habits, family history, and socio-economic status are not recorded in the NHIRD. However, these factors are crucial in the development of atherosclerosis and PAOD. Finally, the NHIRD does not include data on patients' drug regimens, which should be considered for evaluating the risk factors for PAOD.

CONCLUSION

In conclusion, our study revealed that the risk of PAOD was 1.75-fold higher in the patients with leptospirosis compared with the general population. The evidence derived from this retrospective cohort study is generally of a lower methodological evidence than that from the randomized controlled trial because a retrospective cohort study is subject to many biases due to lack of the necessary adjustments for possible risk factors associate with PAOD. Therefore, further investigation with a prospective and randomized controlled study design to reveal the cause-effect between Leptospirosis and PAOD would be worthwhile.

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