Neurofilament light chain is associated with olfactory dysfunction in US adults: findings from National Health and Nutrition Examination Survey

Juanying Zhen¹, Jiayu Huang², Qing Cao³, Bernard Man Yung Cheung^{1,4,5}, Chao Li^{1,4} Rhinology 62: 5, 548 - 556, 2024 https://doi.org/10.4193/Rhin24.001



Abstract

Background: Many individuals who have olfactory dysfunction are not aware of their impairment, which results in delayed detection of potentially hazardous situations. Simple and accurate methods for objectively assessing olfactory function are needed. In this study, we aim to investigate the utility of serum neurofilament light chain (NfL) levels as an indicator of olfactory dysfunction. Methodology: We analysed data on 1290 participants aged 40 years and older, who had valid data on olfaction and NfL level from the National Health and Nutrition Examination Survey 2013–2014. Multivariable modeling was used to investigate the relationship between olfactory dysfunction and NfL. **Results**: Among 1290 participants, 174 participants had olfactory dysfunction based on the results of the NHANES Pocket Smell Test. In ordinal regression models, objective olfactory dysfunction was associated with NfL. After adjusting for age, sex, race/ethnicity, diabetes, smoking, olfaction-related medical history, Parkinson's disease and Alzheimer's disease, the association remained significant. In logistic regression models, compared to participants with lower levels of NfL in the first tertile, those in the second and third tertiles had higher odds of objective olfactory dysfunction. There was no association between self-reported olfactory dysfunction and NfL tertiles. **Conclusions**: A strong association between objective olfactory dysfunction and serum NfL level was observed. NfL, independent of age, is a reliable marker indicating the development of olfactory dysfunction. The measurement of serum NfL level provides valuable support for assessment of olfactory dysfunction in clinical practice.

Key words: smell, public health, olfaction disorders, epidemiologic measurements, diagnostic techniques, respiratory system, NHANES

Introduction

In daily life, many individuals who have olfactory dysfunction are not aware of their impairment. In the United States (US) National Health and Nutrition Examination Survey (NHANES), misidentification rates for warning odours were 20.3 % for smoke and 31.3 % for natural gas among adults \geq 70 years ⁽¹⁾. In the National Institute of Environmental Health Sciences' Sister Study, the sensitivity in reporting poor olfaction was low (22.6%) in middle-aged and older women⁽²⁾. Due to the sense of smell's integral role in our daily life, olfactory dysfunction has been found to decrease life quality and has been associated with mortality ^(3, 4). Unawareness of olfactory dysfunction may cause delayed detection of house fires, gas leaks and toxic fumes⁽⁵⁾. The inconsistency between subjective olfaction and objective olfactory test results indicates the poor sensitivity of self-reported olfaction for diagnosis of olfactory dysfunction. Simple and accurate methods for estimating olfactory function are needed.

Neurofilament light chain (NfL) is a biomarker of neurodegenerative diseases, including Parkinson's disease (PD), Alzheimer's disease (AD) and Huntington's disease (6-8), while olfactory dysfunction is an early symptom of such diseases. NfL, known as neurofilament light polypeptide, is a member of the intermediate filament protein family. It is a subunit of neurofilament, which is structural component of axons ⁽⁹⁾. NfL is released in large quantities following axonal damage in neurodegenerative, inflammatory, vascular and traumatic diseases ⁽¹⁰⁾. Given the fact that NfL is released in response to axonal damage (11), it stands to reason that the damage to the olfactory nerve seen in olfactory dysfunction could produce a measurable increase in NfL levels. Therefore, investigating the relationship between NfL and olfactory dysfunction may provide valuable insights into the pathophysiology of olfactory dysfunction and aid in the development of novel diagnostic and therapeutic strategies for olfactory dysfunction.

In this study, we aimed to investigate the association between olfactory dysfunction and serum NfL levels in a nationally representative population in the US by exploring data from NHANES 2013-2014.

Materials and methods

Database and study population

NHANES is a nationally representative survey designed to assess the health and nutritional status of the civilian noninstitutionalized US population since 1999. The study protocol was approved by the Centers for Disease Control and Prevention (CDC) Institutional Review Board. Written informed consent was obtained from study participants and our study utilised open-access data for analysis. The assessment included interviews, physical examinations and laboratory tests. Interviews were conducted at home by a trained interviewer. Physical examinations and laboratory tests were carried out at well-equipped mobile examination centres in the US. NfL was tested on eligible participants who consented to store their samples for future research. The survey examines a representative sample of about 5,000 noninstitutionalised persons each year, with public-use data released in 2-year cycles. The nationally representative sample was built using stratified, multistage probability sampling method. Details regarding protocols of data collection and sampling methodology can be found on its website: <u>http://www.cdc.gov/ nchs/nhanes.htm</u>.

In this study, data were obtained from the NHANES 2013–2014 cycle. Participants who were pregnant were excluded from the smell exam according to the protocol ⁽¹²⁾. Among the 1298 participants aged 40 years and older who had valid data on NfL level and olfactory dysfunction, 1290 participants who had valid data on diabetes, smoking and olfaction-related medical history were included in our study.

NfL measurement

NfL was measured in serum samples of participants aged 20-75 years from NHANES 2013-2014. The blood samples were frozen at -20° C until they were shipped weekly to the CDC's central laboratory and where they were stored at -80° C. Details of laboratory methodology are available on the NHANES website ⁽¹³⁾.

NfL level was measured by a highly sensitive acridinium ester (AE) immunoassay (Siemens Healthineers) on the fully automated Attelica immunoassay system. Compared with other chemiluminescent technologies, the advantages of AE include high quantum yields, rapid kinetics, hydrophilicity, hydrolytic stability and small size. The analytical process followed strict quality control and quality assurance standards. Quality control samples and additional replicate samples were run each 8-hour shift to ensure the collection of accurate, reliable data.

Olfactory tests

Olfaction was measured using the NHANES Pocket Smell Test (Sensonics, Inc), which is an 8-item scratch and sniff test. Participants were asked to smell eight specific odorants including chocolate, strawberry, smoke, leather, soap, grape, onion and natural gas, in an established sequence. One of the four possible responses should be chosen for each odorant strip according to the forced-choice design. The total number of items that were correctly recognised was used to calculate the overall test score. Participants who had a score of 6 or above (score range, 0-8) were considered to have normal olfaction. Participants who correctly identified 5 odours or less were considered to have olfactory dysfunction ⁽¹⁴⁾. Good test-retest reliability of NHANES olfaction protocol has been supported by previous studies ^(14, 15).

lable 1. Clinical characteristics of all 1290 participants among participants with and without objectively measured olfactory dysfunction

	Total	Normal olfactory function	Olfactory dysfunction	P-value
Ν	1290	1116	174	
Age, (range \geq 40), years	55.2 (0.4)	54.9 (0.4)	58.5 (0.7)	<0.001
Age group, %				0.038
40–64 years	79.3 (1.4)	80.2 (1.5)	71.7 (3.9)	
≥ 65 years	20.7 (1.4)	19.8 (1.5)	28.3 (3.9)	
Male, %	48.5 (1.1)	47.2 (1.2)	59.3 (4.8)	0.037
Race/ethnicity, %				0.228
Non-Hispanic white	69.8 (3.8)	70.9 (3.7)	60.7 (6.2)	
Non-Hispanic black	11.1 (1.9)	10.5 (1.7)	16.2 (3.9)	
Mexican American	7.2 (1.9)	6.8 (1.7)	10.3 (3.8)	
Other	11.9 (1.6)	11.8 (1.7)	12.8 (2.1)	
Diabetes, %	21.2 (1.4)	19.8 (1.4)	32.6 (3.9)	0.003
Smoking, %	47.2 (2.8)	46.8 (3.2)	50.4 (6.0)	0.625
Olfaction-related history, %				
Sinus infection	44.4 (1.5)	46.0 (1.7)	31.7 (4.8)	0.022
Persistent cold symptoms	6.6 (1.1)	6.6 (1.2)	5.8 (1.7)	0.675
Previous head injury	18.0 (1.2)	18.4 (1.2)	14.5 (4.3)	0.446
Nasal or facial fracture	20.2 (2.2)	19.9 (2.2)	22.8 (4.9)	0.511
NfL, pg/ml	20.5 (1.7)	20.1 (1.7)	24.2 (2.4)	0.019

NfL, Neurofilament light chain. Data are expressed as mean or percent (SE).

The NHANES health technicians were trained by expert consultants and survey staff before conducting the olfactory tests. And the performance of technicians was monitored to verify data collection accuracy.

Data on self-reported smell ability was collected in the interview by trained interviewers using the Computer-Assisted Personal Interviewing system. Self-reported olfactory dysfunction was defined to meet one of the following criteria: 1) having a problem with ability to smell, 2) worse sense of smell since 25 years old or 3) phantosmia as previously defined ⁽¹⁶⁾.

Other study measures

Self-reported race/ethnicity was categorised as non-Hispanic whites, non-Hispanic blacks, Mexican Americans and others. Participants who reported lifetime use of \geq 100 cigarettes were considered as smokers. Diabetes was defined as having diabetes diagnosis by a doctor or health professional, or fasting glucose of \geq 7.0 mmol/L (126 mg/dL), or non-fasting glucose of \geq 11.1 mmol/L (200 mg/ dL), or taking diabetic medication. Olfactionrelated history included persistent cold/flu, sinus infections, a loss of consciousness because of a head injury, and a broken nose or other serious injury to the face or skull. Persistent cold/ flu was defined as having a head cold or flu for longer than a month during the past 12 months. Sinus infections were defined as having two or more sinus infections. Participants were considered to have Parkinson's disease (PD) or Alzheimer's disease (AD) if they reported taking medications prescribed for these conditions ⁽¹⁷⁾. PD medications included Benztropine, Carbidopa, Levodopa, Ropinirole, Methyldopa, Entacapone and Amantadine. AD medications included Rivastigmine, Galantamine, Donepezil and Memantine.

Statistical analysis

Analyses were performed using the complex sampling function of SPSS version 28.0 (IBM Corporation, Armonk, NY, USA). P-value \leq 0.05 was considered statistically significant. According to NHANES analytic guidelines, the two-year specific sample weight for serum NfL was used in this study, which accounts for complex survey design, survey non-response, and poststratification adjustment. Clinical characteristics of participants classified according to objective olfaction status (normal olfactory function and olfactory dysfunction) and tertiles of serum NfL level (tertile 1: \leq 11.70 pg/ml, tertile 2: 11.71~19.00 pg/ml and tertile 3: > 19.00 pg/ml) ⁽¹⁸⁾ is shown in Table 1 and Table 2. A natural logarithmic transformation was applied to the data before analysis as the distribution of NfL level significantly deviates from a normal distribution. Linear regression was used Table 2. Clinical characteristics of all 1290 participants according to NfL tertiles.

	Tertile 1 (≤ 11.70 pg/ml)	Tertile 2 (11.71~19.00 pg/ml)	Tertile 3 (> 19.00 pg/ml)	P-value
Ν	438	425	427	
Age, (range \geq 40), years	50.0 (0.5)	56.2 (0.7)	59.4 (0.5)	<0.001
Age group, %				<0.001
40-64 years	93.2 (1.5)	78.9 (2.3)	66.2 (3.0)	
≥ 65 years	6.8 (1.5)	21.1 (2.3)	33.8 (3.0)	
Male, %	46.6 (3.0)	45.0 (2.1)	53.9 (2.0)	0.053
Race/ethnicity, %				0.154
Non-Hispanic white	58.4 (6.0)	75.1 (4.3)	75.5 (4.2)	
Non-Hispanic black	15.3 (3.4)	8.7 (1.8)	9.6 (1.9)	
Mexican American	10.5 (2.4)	5.1 (1.8)	6.2 (1.9)	
Other	15.8 (2.3)	11.2 (2.3)	8.7 (2.0)	
Diabetes, %	13.2 (1.8)	19.9 (1.9)	30.3 (3.0)	0.001
Smoking, %	44.1 (3.3)	46.5 (3.7)	51.0 (3.9)	0.150
Olfaction-related history, %				
Sinus infection	45.4 (3.7)	42.4 (2.1)	45.6 (2.4)	0.473
Persistent cold symptoms	4.8 (1.1)	6.5 (1.7)	8.4 (2.0)	0.081
Previous head injury	15.3 (2.1)	17.2 (2.2)	21.3 (2.0)	0.217
Nasal or facial fracture	18.4 (2.7)	18.3 (3.3)	24.0 (4.0)	0.402
Objectively measured olfactory dys- function, %	7.8 (1.5)	9.6 (1.6)	15.1 (2.4)	0.008
Self-reported olfactory dysfunction, %	17.4 (2.5)	23.7 (3.6)	22.8 (2.5)	0.251

NfL, Neurofilament light chain. Data are expressed as mean or percent (SE).

to investigate the relationship between olfactory dysfunction and NfL level while ordinal regression was used to investigate the relationship between olfactory dysfunction and NfL tertiles. Ordinal regression was also used to investigate the relationship between olfactory test score and NfL level. Age (categorised as 40-64 years and \geq 65 years) ⁽⁴⁾, sex, race, diabetes, smoking and olfaction-related medical history were adjusted in the multivariate models. We further compared participants' age in three NfL groups (Figure 1).

Results

This study included 1290 participants aged 40 years and older from NHANES 2013-2014 (613 men and 677 women; mean [SE] age: 55.2 [0.4] years). All percentages were accounted for a complex, multistage, probability sampling design.

The clinical characteristics of participants are shown in Table 1 and Table 2. Of all the participants inlucded in our study, 174 (13.5%) had olfactory dysfunction and 20.7% of them were 65 years or older. Compared to participants who had normal olfactory function, participants with olfactory dysfunction were more likely to be older (P<0.001), men (P=0.037) and



Figure 1. The mean age of participants in different tertiles in 2013-2014. Means and standard errors are shown. NfL, Neurofilament light chain.

Table 3. Association between	olfactory dysfunction and	neurofilament light chain.
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	Self-reported olfactory dysfunction	Objectively me dysfi	easured olfactory unction
	Ordinal (tertiles), OR (95% CI)	Linear (In-transformed), B (95% CI)	Ordinal (tertiles), OR (95% Cl)
Unadjusted model	1.258 (0.959-1.652)	0.177 (0.033-0.320)	1.788 (1.250-2.556)
Model 1	1.176 (0.910-1.519)	0.188 (0.047-0.329)	1.876 (1.294-2.718)
Model 2	1.159 (0.894-1.503)	0.153 (0.025-0.282)	1.767 (1.267-2.464)
Model 3	1.113 (0.859-1.447)	0.150 (0.024-0.276)	1.760 (1.255-2.468)
Model 4	1.196 (0.924-1.549)	0.125 (0.001-0.250)	1.634 (1.089-2.451)
Model 5	1.164 (0.914-1.481)	0.117 (-0.006-0.241)	1.603 (1.067-2.408)

Tertile 1: \leq 11.70 pg/ml, Tertile 2: 11.71-19.00 pg/ml, Tertile 3: >19.00 pg/ml. Model 1: adjusted for sex and race/ethnicity; Model 2: further adjusted for diabetes and smoking; Model 3: further adjusted for olfaction-related medical history; Model 4: further adjusted for age (40-64 years and \geq 65 years); Model 5: further adjusted for Parkinson's disease and Alzheimer's disease.

Table 4. Association between olfactory test score and In-transformed NfL.

	OR	95% Cl	P-value
Unadjusted model	1.313	1.058-1.630	0.017
Model 1	1.355	1.104-1.662	0.006
Model 2	1.328	1.052-1.677	0.020
Model 3	1.329	1.059-1.669	0.018
Model 4	1.248	1.008-1.546	0.043
Model 5	1.233	1.000-1.520	0.050

NfL, neurofilament light chain. Model 1: adjusted for sex and race/ethnicity; Model 2: further adjusted for diabetes and smoking; Model 3: further adjusted for olfaction-related medical history; Model 4: further adjusted for age (40-64 years and \geq 65 years); Model 5: further adjusted for Parkinson's disease and Alzheimer's disease.

diabetic (P<0.001). The mean (SE) NfL level was 24.2 (2.4) pg/ ml for patients with olfactory dysfunction and 20.1 (1.7) pg/ml for participants with normal olfactory function. From NfL tertile 1 to tertile 3, there is an increase in average age from 50.0 to 59.4 years (Figure 1), diabetes prevalence from 13.2% to 30.3% and olfactory dysfunction prevalence from 7.8% to 15.1% (P for age<0.001, diabetes=0.001 and olfactory dysfunction=0.008).

We found an association between olfactory dysfunction and NfL (Table 3). In linear regression models, olfactory dysfunction was associated with In-transformed NfL (B= 0.177; 95% CI=0.033-0.320; P=0.019). After adjusting for sex, race/ethnicity, diabetes and smoking, the association remained significant (B=0.153; 95% CI=0.025-0.282; P=0.038). The association was significant consistently after further adjustment for olfaction-related history (B= 0.150; 95% CI=0.024-0.276; P=0.022). The association still existed after adjusting for age (B= 0.125; 95% CI=0.001-0.250; P=0.049). After adjusting for PD and AD, the association was no

longer significant (B=0.117; 95% CI=-0.006-0.241; P=0.061). In ordinal regression models, olfactory dysfunction was associated with NfL tertiles (OR=1.788; 95% CI=1.250-2.556; P=0.003). After adjusting for sex, race/ethnicity, diabetes and smoking, the association remained significant (OR=1.767; 95% CI=1.267-2.464; P=0.002). The association was significant consistently after further adjustment for olfaction-related history (OR=1.760; 95% CI=1.255-2.468; P=0.003). The association still existed after adjusting for age (OR=1.634; 95% CI=1.089-2.451; P=0.021). After adjusting for PD and AD, the association remained significant (OR=1.603; 95% CI=1.067-2.408; P=0.026). There was no association between self-reported olfactory dysfunction and NfL tertiles in any of the models.

The association between olfactory test score and In-transformed NfL is shown in Table 4. Ln-transformed NfL was associated with olfactory test scores (OR=1.313; 95% CI=1.058-1.630; P=0.017). After adjusting for sex, race/ethnicity, diabetes and smoking,

the association remained significant (OR=1.328; 95% CI=1.052-1.677; P=0.020). The association was significant consistently after further adjustment for olfaction-related history (OR=1.329 95% CI=1.059-1.669; P=0.018). The association still existed after adjusting for age (OR=1.248; 95% CI=1.008-1.546; P=0.043). Higher serum NfL level was associated with a higher risk of olfactory dysfunction (tertile 3 vs tertile 1, OR=2.13; 95% CI=1.39-3.26; P for trend=0.008, Supplementary Table 1). After adjusting for sex, race/ethnicity, diabetes, smoking and olfaction-related history, the association remained significant (tertile 3 vs tertile 1, OR=2.12; 95% CI=1.41-3.19; P=0.007). The association still existed after adjusting for age (tertile 3 vs tertile 1, OR=1.97; 95% CI=1.25-3.09; P=0.027). After adjusting for PD and AD, the association remained significant (tertile 3 vs tertile 1, OR=1.89; 95% CI=1.19-3.00; P=0.043). Sensitivity and specificity for NfL levels indicating olfactory dysfunction are shown in Supplementary Table 2.

Discussion

In this nationally representative study, we found that objectively measured olfactory dysfunction was associated with elevated serum NfL level in adults aged 40 years and older. The association remained significant after adjusting for sex, race/ethnicity, diabetes, smoking, olfaction-related medical history, age, PD and AD, which suggested that NfL is an independent indicator of objectively measured olfactory dysfunction. There was no association between self-reported olfactory dysfunction and NfL level.

To our knowledge from the literature review, this is the first study to elucidate the relationship between NfL and olfactory dysfunction. Previous studies have consistently shown that self-reported olfactory function has poor sensitivity. In a study involving 1468 US adults aged 57-85 years, 12.4% of the participants reported experiencing poor olfactory function, but objective tests revealed that 22.0% actually had olfactory dysfunction ⁽¹⁹⁾. It was estimated that only one-fourth of participants with olfactory dysfunction accurately reported their impairment, demonstrating the low sensitivity of self-reported olfaction (19). The limited accuracy of self-awareness regarding olfactory dysfunction was further supported by a large-scale cohort study involving over 40 thousand participants ⁽²⁾. These findings indicate that a significant portion of individuals with olfactory dysfunction are not aware of their impairment. Selfreported olfactory dysfunction may not be a reliable indicator of actual impairment in the general population. Relying solely on self-reported symptoms is imprecise and can lead to missed diagnoses and delayed treatment. Furthermore, it may result in failure to identify life-threatening situations such as gas leaks or fires, leading to serious consequences ⁽⁵⁾. Our study provides a feasible method to evaluate olfactory dysfunction.

Objective olfactory testing is often used in the department of otolaryngology rather than other departments due to the requirement for specialized equipment. Smell Identification Test requires not only proper environmental storage for the sensitive test strips but also attention to the expiration dates. However, to identify the olfactory status of patients is crucial to evaluate other serious neurological disorders. Measurement of NfL eliminates the need for storage and handling steps, and can also be used in some simple physical examinations or primary care clinic setting. It can even be done alongside other blood tests that may be required, without the need for any additional steps. Combined with evidence of patient's history and physical examination, NfL level could serve as an ideal tool to assess olfactory function. This approach promotes early consultation with relevant departments and provides timely interventions for patients with olfactory dysfunction.

Our findings may establish enhanced utility by measuring NfL in patients already diagnosed with olfactory dysfunction in several ways. Firstly, measuring NfL level provides additional information in understanding the underlying causes of olfactory dysfunction and monitoring the progression of neurodegenerative diseases. Secondly, elevated NfL level in patients with olfactory dysfunction suggest that their smell impairment may be due to an ongoing neurodegenerative process. This information is useful in guiding further diagnostic testing and therapeutic interventions. Thirdly, longitudinal assessment of NfL level in patients with olfactory dysfunction and neurodegenerative diseases provides insights into the progression of the disease and the effectiveness of therapeutic interventions.

Our study revealed that NfL, independent of age, is an indicator of olfactory dysfunction. Epidemiological studies have shown that olfactory function declines with age, with the prevalence of dysfunction increasing from 10% at the age of 60 to around 60% at the age of 90 $^{(20-22)}$. The decline of olfactory function starts at age of 50 years in healthy humans (23). The aging process contributes to neural degeneration that is related to deterioration in olfactory function. Additionally, healthy aging individuals have been found to have increased level of serum NfL. In a cross-sectional study involving 359 healthy participants, reference value for NfL level was estimated for their specific study by its authors and cerebrospinal fluid NfL level were found to be strongly associated with age ⁽²⁴⁾. Compared with their normal upper reference value for NfL level at the age of 20 years, NfL level increased by 3.4-fold at the age of 60 years ⁽²⁴⁾. Our study also observed a gradual increase in serum NfL level with age (Figure 1). Despite the potential effect of aging on olfactory function and NfL level, our study found that the association between NfL and olfactory dysfunction remained significant after adjusting for age. It means that NfL is an age-independent biomarker for olfactory

dysfunction. Therefore, NfL not only reflects the effects of aging but also indicates axonal damage in olfactory dysfunction.

In our analysis, we adjusted for relevant cofounders to account for their potential influence on olfactory dysfunction. Older age, male sex and upper respiratory infections were considered as major risk factors for olfactory dysfunction. Meta-analysis has revealed the racial disparities in olfactory dysfunction, suggesting that Blacks have higher prevalence of olfactory dysfunction compared to Whites ⁽²⁵⁾. Epidemiological studies have found that patients with diabetes have decreased olfactory acuity (26). Smoking is commonly considered a risk factor for poor olfaction ⁽²⁷⁾. Besides, sinus infection is a common cause of smell loss ⁽²⁸⁾. Head injury, particularly nasal or midface fractures, has been found to be associated with olfactory dysfunction, as it may result in injury to the olfactory tract ⁽²⁹⁾. Nasal bone or midface fractures can also lead to disruption of the normal airflow and prevent odorants from reaching the olfactory nerve, causing conductive loss of smell. Olfactory dysfunction is an early symptom for PD and AD.

There were several possible mechanisms underlying the association between NfL and olfactory dysfunction. Sinonasal disease, upper respiratory tract infection and traumatic brain injury are three primary factors that can contribute to olfactory dysfunction ⁽³⁰⁻³²⁾. Traumatic brain injury could result in the damage of olfactory cortex (32, 33), leading to an increase in NfL level. In sinonasal diseases and upper respiratory tract infection, the pathogen may enter into the central nervous system (CNS) and cross the blood-brain-barrier (BBB) through transcellular transportation, paracellular transportation and the Trojan-horse mechanism, which follows increased permeability of the BBB ⁽³⁴⁻³⁶⁾. The breakdown of BBB results in reduced removal of waste and increased infiltration of immune cells which can cause disruption of glial and neuronal cells ^(37, 38). Besides, toxins may enter into CNS following the breakdown of BBB, which causes neural injury ⁽³⁹⁾. Finally, the neural damage is followed by an increase in NfL level. These possible mechanisms provide a pathophysiological basis for the association between NfL and olfactory dysfunction.

Strengths and limitations

The present study has several strengths. Firstly, a large sample size that represents the general US adult population was used. The 1290 participants in this study represent 124,297,018 US citizens. Secondly, NHANES Pocket Smell Test was used by trained examiners to assess participants' olfactory function. This method has been considered as a rapid and accurate method for detection of olfactory dysfunction ^(15, 40). Thirdly, potential confounders

including sex, race/ethnicity, diabetes, smoking, olfactionrelated medical history and age were adjusted to confirm the association between olfactory dysfunction and NfL.

There are limitations in our study. Firstly, only 7.8% in tertile 1, 9.6% in tertile 2, and 15.1% of participants in tertile 3 had olfactory dysfunction. The vast majority of participants in every tertile of NfL did not have olfactory dysfunction. The measurement of serum NfL level acts as a screening method of olfactory dysfunction in general practice. If NfL level is elevated, objective olfactory testing should be further used to confirm the diagnosis. Secondly, due to the nature of cross-sectional design, it inevitably avoids determining the causal relationship between olfactory dysfunction and NfL. NfL has a strong association with neuronal damage ⁽¹⁰⁾, so it is reasonable to understand that olfactory dysfunction results in higher serum NfL level. Thirdly, we took medication use as an indication of the most common neurodegenerative diseases, PD and AD in the regression model as confounders. Neurodegenerative diseases might be underestimated due to unawareness of early symptoms. However, the symptoms of PD tend to be noticeable, allowing medication use to largely reflect disease status. The use of AD medications indicates severe disease or symptoms.

Conclusion

In this nationally representative study, a strong association between olfactory dysfunction and serum NfL level was observed. This finding suggests that NfL, independent of age, is a reliable marker indicating the development of olfactory dysfunction. The measurement of serum NfL level provides valuable support for assessment of olfactory dysfunction in clinical practice.

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Authors' contributions

Concept and design: CL, JZ; Acquisition, analysis, or interpretation of data: JZ and CL; Drafting of the manuscript: JZ; Critical revision of the manuscript for important intellectual content: CL, BMYC, JH, QC; Supervision: CL, BMYC.

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Conflicts of interest

The authors declare no conflicts of interest.

Data availability

None to declare.

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Chao Li

Department of Medicine School of Clinical Medicine The University of Hong Kong Queen Mary Hospital Pokfulam Hong Kong SAR

E-mail: dcli@connect.hku.hk ORCID: 0000-0002-7543-3973 Bernard Man Yung Cheung Department of Medicine School of Clinical Medicine The University of Hong Kong Queen Mary Hospital Pokfulam Hong Kong SAR

E-mail: mycheung@hku.hk ORCID: 0000-0001-9106-7363

Juanying Zhen¹, Jiayu Huang², Qing Cao³, Bernard Man Yung Cheung^{1,4,5}, Chao Li^{1,4}

¹ Department of Medicine, School of Clinical Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong SAR January 3, 2024

² Department of Otorhinolaryngology Head and Neck Surgery, Huizhou Central People's Hospital, Huizhou, China

³ Allergy Center, Department of Otolaryngology, Affiliated Eye and ENT Hospital, Fudan University, Shanghai, China

⁴ State Key Laboratory of Pharmaceutical Biotechnology, The University of Hong Kong, Pokfulam, Hong Kong SAR

⁵ Institute of Cardiovascular Science and Medicine, The University of Hong Kong, Hong Kong SAR

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SUPPLEMENTARY MATERIAL

	Unadjusted model	Model 1	Model 2	Model 3	Model 4*	Model 5
Tertile 1	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Tertile 2	1.23 (0.70-2.15)	1.39 (0.80-2.40)	1.35 (0.77-2.38)	1.32 (0.76-2.31)	1.27 (0.74-2.18)	1.32 (0.77-2.26)
Tertile 3	2.13 (1.39-3.26)	2.31 (1.50-3.55)	2.12 (1.40-3.22)	2.12 (1.41-3.19)	1.97 (1.25-3.09)	1.89 (1.19-3.00)
P for trend	0.008	0.005	0.008	0.007	0.027	0.043

Supplementary Table 1. Association between objective olfactory dysfunction and NfL tertiles.

Values are weighted odds ratio (95% confidence interval). Model 1: adjusted for sex and race. Model 2: further adjusted for diabetes and smoking. Model 3: further adjusted for olfaction-related medical history including recent cold symptoms, previous sinus infection, previous head injury, and nasal or facial fracture. Model 4: further adjusted for age. Model 5: further adjusted for Parkinson's disease and Alzheimer's disease. *age was categorized as two groups: 40-64 years and \geq 65 years. Tertile 1: \leq 11.70 pg/ml, Tertile 2: 11.71-19.00 pg/ml, Tertile 3: >19.00 pg/ml.

Supplementary Table 2. Sensitivity and specificity for NfL level indicating olfactory dysfunction.

NfL level	Sensitivity	Specificity
7.25 pg/mL	90.6%	8.6%
9.85 pg/mL	81.0%	21.6%
12.75 pg/mL	70.8%	40.4%

NfL, Neurofilament light chain.