



# Prevalence of Multiple Sclerosis in a Turkish City Bordering an Iron and Steel Factory

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**Background and Purpose** Multiple sclerosis (MS) is an autoimmune disease characterized by inflammatory demyelination. Recent studies have shown that long-term exposure to air pollutants (including PM<sub>10</sub> particulates) is potentially an environmental risk factor for MS. We aimed to determine the prevalence rates of MS in two cities with different levels of air pollution.

**Methods** This door-to-door population-based study was conducted between April 2014 and June 2015. Two cities were screened for the prevalence rates of MS: 1) Karabük, which borders an iron-and-steel factory, and 2) Akçakoca, which is a coastal city located in the same region. A validated survey form was used for screening MS. The 2010 McDonald Criteria were used for diagnosing MS. The patients were examined twice, first by a neurology assistant in the field and then by a senior neurologist in public health centers in the cities.

**Results** The prevalence of MS was 95.9/100,000 in Karabük and 46.1/100,000 in Akçakoca. In total, 33 patients were diagnosed with clinically definite MS. The female/male ratio was 1.5, and 21 patients were diagnosed with relapsing-remitting MS, 9 with secondary progressive MS, and 3 with primary progressive MS.

**Conclusions** We found that the prevalence of MS was more than two fold higher in Karabük than in Akçakoca, which supports a link between air pollution and the pathogenesis of MS. However, larger etiological and epidemiological studies are needed to confirm this hypothesis.

**Key Words** multiple sclerosis, prevalence, air pollution, Turkey.

## INTRODUCTION

Multiple sclerosis (MS) is a demyelinating immune-mediated disease of the central nervous system (CNS), and is the most common cause of disability in young people in the developed world.<sup>1</sup> The most striking epidemiological characteristic of MS is its uneven geographical distribution. Meta-analyses of studies on MS etiology has revealed almost universal increases in the prevalence and incidence over time.<sup>2</sup> The prevalence and incidence rates of MS have also increased dramatically in the Mediterranean region over the last few decades.<sup>3,4</sup>

Our recent studies performed in the Black Sea region have revealed a wide range in the prevalence rate of MS, from 18.6/100,000 in Artvin in the east of the region, to 101.3/100,000 in Istanbul.<sup>5,6</sup>

A possible association between MS and air pollution has been reported,<sup>7-9</sup> as has a likely association between MS and trace metals (e.g. zinc and heavy metals) in sewage and river water.<sup>10-12</sup>

This study was conducted after a local neurologist raised concerns over the perceived excessive prevalence of MS in Karabük, which is located close to a factory. Data from the

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national air quality monitoring authority indicate that the prevalence of air pollution in this region is well known and well documented. A recent study that analyzed lichens and plants found that the concentration of trace metals is high in Karabük.<sup>13</sup>

The goal of the present study was to calculate the prevalence rates of MS in Karabük and Akçakoca both separately and combined. We aimed to determine whether the potential exposure to air pollution associated with living in Karabük is linked to an increased prevalence of MS. Such a finding might indicate that air pollution impacts the development of this disease.

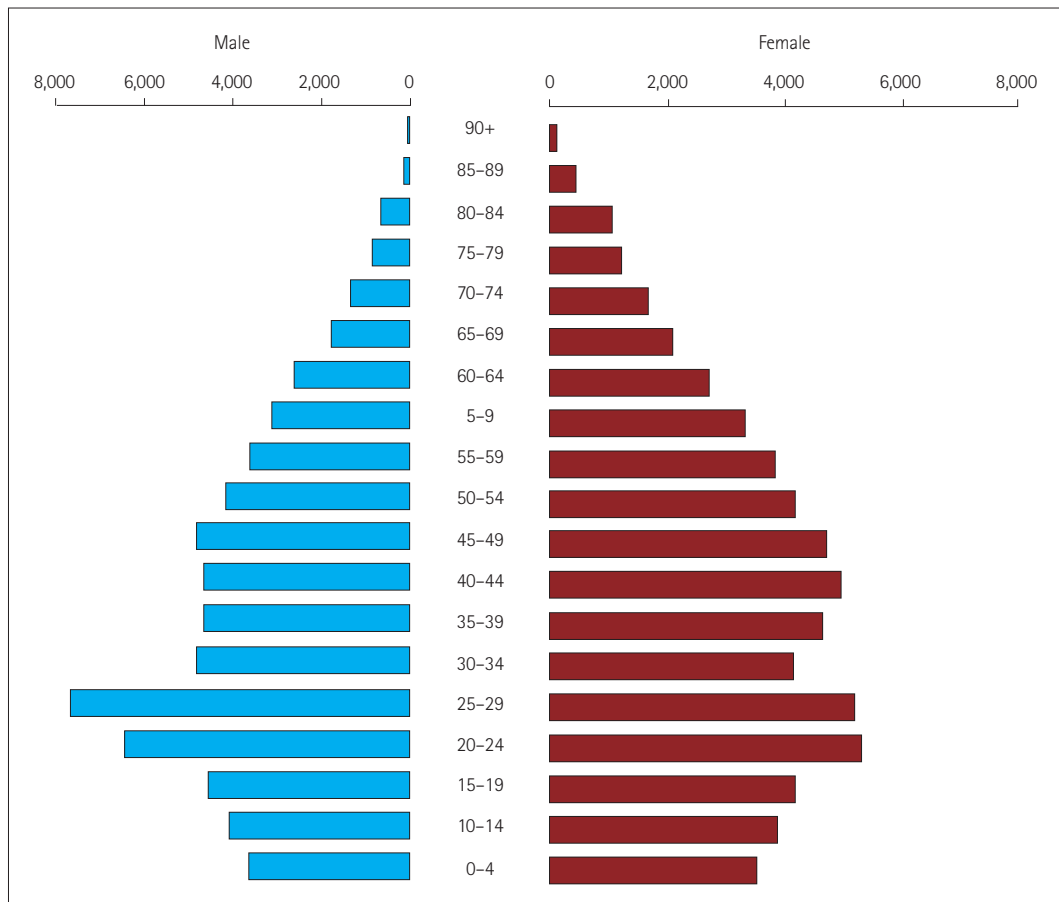
## METHODS

We conducted descriptive, cross-sectional, door-to-door surveys in a joint effort with neurologists from Dr. Lütfi Kırdar Kartal Training and Research Hospital and public health specialists from Marmara University. The study was approved by the Local Ethics Committee of Dr. Lütfi Kırdar Kartal Training and Research Hospital (Approval No. 89513307/1009/233).

## Characteristics of Karabük

Karabük is a city in the western Black Sea region located at latitude 40.08°N and longitude 32.04°E. The city is at an altitude of 280 m and covers an area of 760 km<sup>2</sup>. Most of its surface is covered by trees. In winter it is cold and snowy while in summer it is hot, and the mean annual rainfall is 780 mm. The population of Karabük is highly homogeneous, comprising mainly Turkish people, and there is little migration. Most people are laborers, with most households including a worker at the local iron-and-steel factory or a retiree from that factory. Farmers constitute a small proportion of the population. The mean gross domestic product (GDP) per capita in Karabük was US\$ 5,800 in 2014, and the mean duration of education is 6.7 years. There are 6 hospitals and 234 specialist doctors in the province. There is easy access to hospitals, specialists, and magnetic resonance imaging (MRI) devices. Karabük was built in the 1930s as the seat of the iron and steel industry in Turkey. There are also chemical plants that produce sulfuric acid and phosphates, and the Zonguldak coal fields are nearby.

One of the main steel producers in Turkey, named Karmir (Karabük Iron and Steel Works), is located in Karabük.



**Fig. 1.** Population pyramid for Karabük.

It has been in operation continuously since being constructed in 1937, and is the main source of local air pollution. According to the 2014 census, the population of the city center was 132,658.<sup>14,15</sup> The population pyramid for Karabük is shown in Fig. 1.

**Characteristics of Akçakoca**

Akçakoca is a small city located 100 km from Karabük and at the same latitude, and the racial and geographical characteristics of the two cities are similar. Akçakoca was chosen for inclusion in this study because it does not have any factories. Akçakoca is a coastal town in the western Black Sea region located at latitude 41.05°N and longitude 31.07°E. It has a 35-km-long coastline along the Black Sea and covers an area of 462 km<sup>2</sup>. Two-fifths of its surface is covered by trees, most of which are nut trees. In winter it is rainy while in summer it is mild, and the mean annual rainfall is 990 mm. Like Karabük, the population of Akçakoca is highly homogenous, comprising mainly Turkish people and with little migration. Most of the population grows plant nuts, while some of the inhabitants are fishermen. The mean GDP per capita in Akçakoca was US\$ 6,500 in 2014 and the mean duration of education is

6.4 years. There are 8 hospitals and 312 specialist doctors in the province, with these facilities reachable within 20 minutes by all of the populace. There is easy access to hospitals, specialists, and MRI devices. Few local tourists visit Akçakoca in the summer, and there are no official reports of air pollution causing health problems. According to the 2014 census, 24,161 people lived in Akçakoca.<sup>15,16</sup> The population pyramid for Akçakoca is shown in Fig. 2. Geographical locations of Karabük and Akçakoca in Turkey map are shown in Fig. 3.

**Screening forms**

We used validated screening forms that we had prepared for our prevalence study conducted in Maltepe, Istanbul in 2002.<sup>5</sup> The questionnaires comprised 26 questions in 2 sections. The first section assessed knowledge about MS, while the addressed MS symptoms. The questions enquired about the presence of present and past symptoms, motor and sensory dysfunction, walking ability, sphincter control, sleep, balance disturbances, ataxia, depression, increased fatigability, and memory disturbances. The teams conducting the field interviews received practical training. The interviewers were informed about the steps of the procedure that were to be

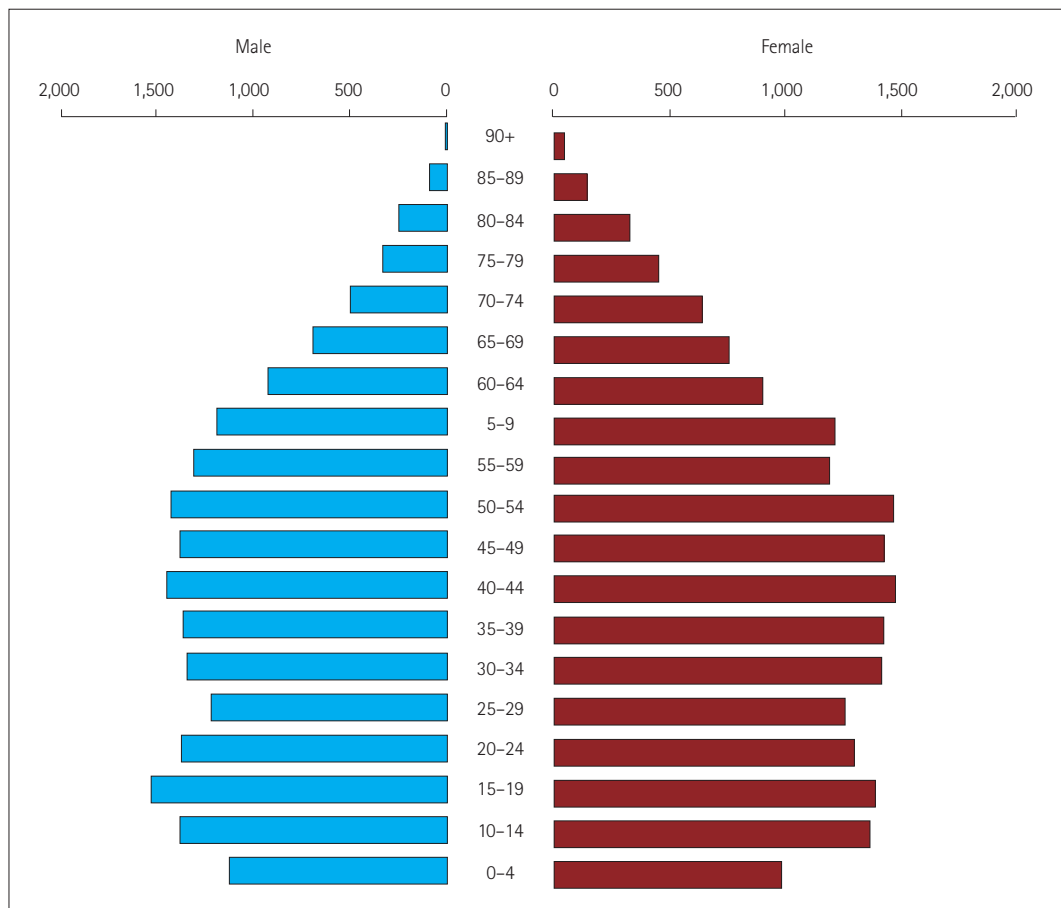
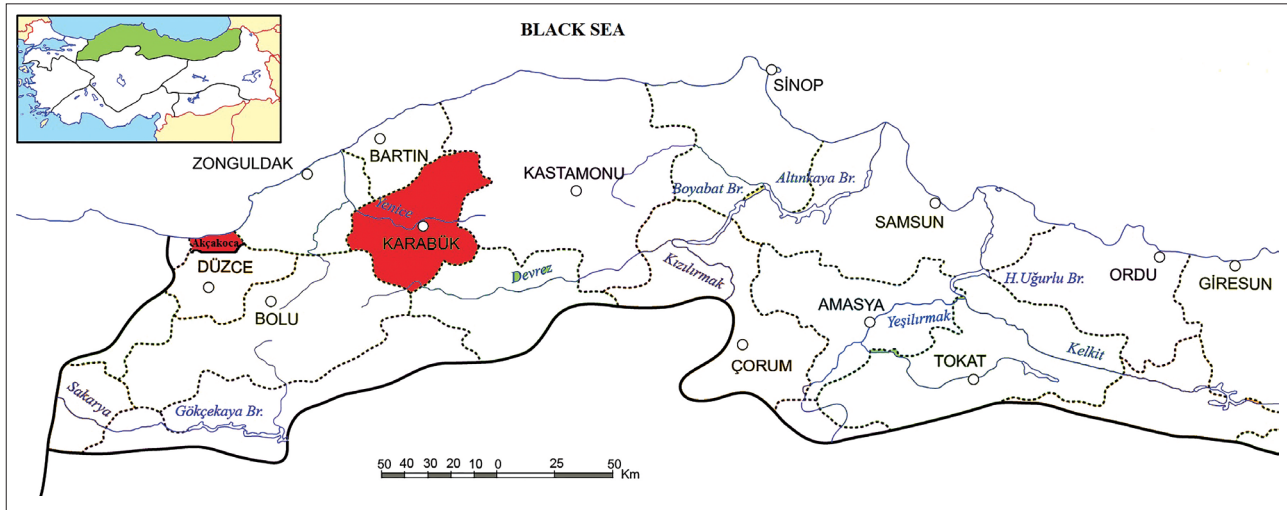


Fig. 2. Population pyramid for Akçakoca.



**Fig. 3.** Geographical locations of Karabük and Akçakoca.

followed; that is, which doors to knock on, what to do when nobody is home, the methods of intervention, how to present the questions, and how to fill in the forms.

### Establishment of the neuroepidemiological field teams and the study procedure

Based on the population of Karabük city center being 132,658,<sup>15</sup> the required sample was calculated as 29,790 for a confidence level of 95% and a margin of error of 0.5%. We therefore visited every fourth household. The houses to be visited were determined beforehand, and in cases where no one was at home, the same house would be visited later. The study was conducted in Karabük between April 1 and 30, 2014.

Based on a population of 24,161 for Akçakoca,<sup>15</sup> the required sample was calculated as 14,833 for a confidence level of 95% and a margin of error of 0.5%. We therefore visited every second household. The houses to be visited were determined beforehand, and in cases where no one was at home, the same house would be visited later. The study was conducted in Akçakoca between May 25 and June 24, 2015.

In order to encourage participation in both cities, an announcement was made at a public health center and at a council meeting 1 week prior to the study commencement to inform the citizens.

Four teams of six persons each (a neurology assistant, a nurse, and four interviewers per team) were established in order to obtain reliable and precise data with a correct provisional diagnosis. The teams were provided with both the theoretical and practical training by neurologists and public health specialists for 2 days on aspects. The teams conducted their visits between 8:30 a.m. and 5:00 p.m. both on weekdays and over the weekend.

Interviews with children or elderly where there were com-

munication difficulties were performed with the help of the persons responsible for their care. The field study lasted approximately 3 weeks.

### The field study

The field study comprised two phases:

- 1) The questions were first asked directly and the questionnaires were completed. Persons who answered affirmatively to one or more of the questions in the section about MS symptoms were examined by the neurology assistant in the team.
- 2) Persons who showed signs of having MS were subsequently re-examined by an experienced neurologist. Those unable to come to their local hospital were examined in their homes.

### Diagnosis

The previous medical results of patients including their MRI images were studied. All laboratory and examination findings were recorded. If needed, further investigations (e.g. immunological screening or MRI) were conducted at a local hospital. The McDonald Criteria were used for diagnosing of MS.<sup>17</sup> Kurtzke's Expanded Disability State Score (EDSS) was used to assess the level of neurological impairment.<sup>18</sup> The medical records of patients diagnosed with MS were checked at the local hospital and compared with the screening results.

### Statistical methods

SPSS software (version 11.5, SPSS Inc., Chicago, IL, USA) was used for calculating frequency distributions and percentages. Minitab statistical software (version 16, SPSS Inc.) was used for calculating the Poisson confidence intervals and comparing prevalence rates.

**Table 1.** Air pollutants values of Karabük city by years and WHO threshold values

Air pollutants	2008	2009	2010	2011	2012	2013	2014	2015	2016	WHO
PM <sub>10</sub> (µg/m <sup>3</sup> )	183	214	153	98	76	83	91	158	84	20
SO <sub>2</sub> (µg/m <sup>3</sup> )	57	57	53	31	20	60	87	121	50	20
NO <sub>2</sub> (µg/m <sup>3</sup> )	-	-	-	-	101	120	123	68	69	40

NO<sub>2</sub>: nitric oxide, PM: particulate matter, SO<sub>2</sub>: sulphur dioxide, WHO: World Health Organization.

## RESULTS

### Findings for Karabük

In total, 29,586 inhabitants of Karabük were visited, and 28,127 answered the questionnaires (1,459 declined to participate). Neurological examinations revealed suspected MS in 31 people. One female patient was diagnosed by our team. She was 22 years old and had experienced two clinical attacks: the first was optic neuritis and in the second she had sensorial and autonomic symptoms. Her EDSS was 2.5. MRI was performed in Karabük State Hospital: cranial MRI revealed five or six T2-weighted hyperintense periventricular lesions, and her spinal MRI findings were normal.

In total, 27 patients were diagnosed with clinically definite multiple sclerosis (CDMS). Other suspected cases were diagnosed with migraine, dementia, normal pressure hydrocephalus, and posttraumatic encephalomalacia. There were 17 patients diagnosed with relapsing-remitting multiple sclerosis (RRMS), 7 with secondary progressive multiple sclerosis (SPMS), and 3 with primary progressive MS. The female/male ratio was 1.2 (15 females). The patients were aged 39.5±10.2 years (mean±SD), their age at disease onset was 31.1±9.6 years, and the EDSS was 3.37±1.70. Nineteen patients were receiving immunomodulatory treatments and two had a family history of MS.

Cerebrospinal fluid (CSF) examinations were performed on 18 patients, of which 12 had a positive oligoclonal band (OCB). Visual evoked potentials (VEP) were measured in 16 patients, and 12 showed prolonged P100 latency. Somatosensory evoked potentials were measured in 12 patients of whom 5 displayed abnormal results. Brainstem auditory evoked potentials (BAEP) were measured in 12 patients, with abnormal results found in 6.

Cranial and spinal MRI was performed in 27 patients. All of them exhibited demyelinating lesions in the brain that fulfilled the 2010 McDonald Criteria. Two patients had cervical spinal demyelinating lesions; these patients were negative for the aquaporin-4 antibody.

The raw prevalence rate of MS in Karabük was 95.9/100,000 and it was 107.1/100,000 after age standardization.

Table 1 presents air pollution data (PM<sub>10</sub>, SO<sub>2</sub>, and NO<sub>2</sub>) from the National Air Pollution Monitoring Network System for Karabük city and also the World Health Organization

**Table 2.** Trace elements values from the area surrounding factory site and the control area

Trace elements	Value (µg/g)	Control (µg/g)
Zn	53.802	28.640
Cu	4.560	1.810
Mn	161.922	44.805
Fe	3,173.40	1,337.50
Pb	9.750	4.000
Ni	8.668	1.162
Cr	4.650	1.694
Cd	0.875	0.733

(WHO) normal threshold values. The trace-element values in the area surrounding the factory as well as in the control area 30 km away are listed in Table 2.

### Findings for Akçakoca

In total, 13,793 inhabitants of Akçakoca were visited, and 12,992 completed the screening form (803 declined to participate). Neurological examinations revealed suspected MS in ten people. A 15-year-old female patient had blurred vision and a urinary emergency. Her complaint had begun 1 month previously. Her brain MRI findings were normal, and she was diagnosed with optic neuritis. She was not diagnosed with MS because she did not fulfill the 2010 McDonald Criteria. The other suspected three cases were diagnosed with Chiari I malformation, migraine, and Behçet's disease; they were also not diagnosed with MS.

Six patients were diagnosed with CDMS. One patient was male (female/male ratio of 5.0), and four patients had RRMS while two had SPMS. The patients were aged 47.5±9.9 years, they had a disease duration of 17.8±7.7 years, their age at disease onset was 32.2±4.5 years, and the EDSS was 4.4±4.0. Two patients were taking immunomodulatory drugs.

The CSF was analyzed in two patients, which produced normal findings. VEP, BAEP, and aquaporin-4 antibody results were not obtainable.

Cranial MRI was performed in all of the patients, which revealed multiple demyelinating lesions in all of them. Their cranial MRI findings supported an MS diagnosis according to the 2010 McDonald Criteria. Cervical MRI was performed in two patients, for which the findings were normal.

The raw prevalence rate of MS in Akçakoca was 46.1/

**Table 3.** Demographic characteristics of the 33 patients

Number of cases	n=33
Female/male	20/13
Age, y, mean±SD	40.2±11.3
Onset of disease, y, mean±SD	30.8±9.1
Duration of disease, y, mean±SD	9.7±5.2
EDSS, mean±SD	3.5±1.8
DMD's treatment	21
Annual attacks, mean±SD	2.3±1.5
Family history	2

DMD's: disease modifying drugs, EDSS: Expanded Disability State Score.

**Table 4.** Initial symptoms of the 33 patients

Symptoms	n (%)
Pyramidal	9 (27.2)
Cerebellar	4 (12.1)
Brainstem	10 (30.3)
Sensorial	12 (36.3)
Autonomic	2 (6.0)
Visual	15 (45.4)
Mental	3 (9.1)

100,000, it was found to be 41.5/100,000 after age standardization.

### Summary

In Karabük and Akçakoca collectively, 43,379 people were visited and 41,119 of them completed the questionnaire form (the other 2,262 persons declined to take part in the study). CDMS was diagnosed in 33 people. Their demographical and clinical characteristics are presented in Table 3, their initial symptoms of are shown in Table 4, and their laboratory findings are summarized in Table 5.

## DISCUSSION

This study found that the overall prevalence rate of MS was 95.9/100,000 in Karabük and 46.1/100,000 in Akçakoca. After age standardization there was a difference between the two regions of more than twofold: 107.1/100,000 and 41.5/100,000, respectively.

This large difference is especially noteworthy given that this study was conducted during the same period using the same diagnostic criteria and with two populations that had similar racial and demographic characteristics.

The genetic characteristics of the inhabitants of the two cities are similar because they come from the same Cepni branch of the Oğuz Turks. Also, the similarity in the age structures of the city populations can be seen when comparing their population pyramids. The mean GDP, level of the education, and

**Table 5.** Laboratory findings of the patients

Tests	n	Positive results	Negative results
Brain MRI	33	33	0
Spinal MRI	29	2	27
CSF OCB	20	12	8
Aquaporin-4	2	0	2
VEP	16	12	4
SEP	12	5	7
BAEP	12	6	6

BAEP: brainstem auditory evoked potential, CSF: cerebrospinal fluid, MRI: magnetic resonance imaging, OCB: oligoclonal band, SEP: somatosensory evoked potential, VEP: visual evoked potential.

health-care availability are very similar in two cities. Moreover, the vitamin D levels and sun exposure in these two cities should be similar because they are located in the same region and at the same latitude and they have similar weather conditions. However, the lack of data prevents statistical comparisons.

In our previous studies we found prevalence rates of 18.6/100,000 in Artvin, 55.6/100,000 in Ordu, and 53.2/100,000 in Erbaa.<sup>6,19</sup> These three cities are also located in the Black Sea region and at the same latitude as Karabük. The prevalence rate in Karabük found in this study was clearly much higher than in those three cities, while the rate in Akçakoca was similar to those Ordu and Erbaa. A highly plausible reason for the much higher prevalence in Karabük is the city being bordering an iron-and-steel factory. It is well documented that a considerable amount of air pollution has been emitted from this factory over a long time period,<sup>20</sup> in contrast, there is no industrialization that could cause air pollution in Akçakoca.

National Air Pollution Monitoring Network System permanent stations are set up in areas of Turkey where air pollution is suspected by mobile stations. There is no permanent observation station in Akçakoca, and hence this city can be considered an area without marked air pollution.

Karabük borders an iron-and-steel factory that has been in operation since 1925. This factory is known to cause air, water, and soil pollution. According to data from the National Air Pollution Monitoring Network System, the levels of air pollutants PM<sub>10</sub>, SO<sub>2</sub>, and NO<sub>2</sub> at Karabük are much higher than the WHO thresholds for health concerns.<sup>20</sup>

A recent study conducted in Karabük assessed the concentration of the trace elements in lichen, which accumulate any such elements that are present in the atmosphere. Much higher concentrations of Cd, Cr, Cu, Fe, Pb, Ni, Mn and Zn were found in the areas surrounding the iron-and-steel factory than in the control area.<sup>13</sup> Another study conducted in Karabük by the same study group showed that the accumula-

tion of trace elements caused DNA variation in lichens and that this effect was great in the sample lichens that had been exposed for 6 months.<sup>13,21</sup>

Few studies worldwide have investigated the relationship between air pollution and the geographical distribution of MS. A recent study in Tehran found significant spatial correlation between the clustering of MS cases and the patterns of PM<sub>10</sub>, SO<sub>2</sub>, and NO<sub>2</sub> concentrations.<sup>22</sup> A study conducted in Finland found that the ambient air quality and especially the concentrations PM<sub>10</sub>, CO<sub>2</sub>, and acidic gases [nitrogen oxides (NO<sub>x</sub>), and SO<sub>2</sub>] was associated with the prevalence of MS in southern Finland. Those authors found that MS relapses were directly associated with the highest level of pollution.<sup>23</sup> A study from Lombardy supported the hypothesis that air pollution plays a role in determining MS occurrence and relapses. By dividing PM<sub>10</sub> concentrations into quartiles, those authors found a dose-response increase in the number of hospital admissions.<sup>24</sup> A study from Strasbourg found that PM<sub>10</sub> exposure appears to be a trigger for MS relapses.<sup>25</sup>

Particulates have been identified both in human brain capillaries and in the brain parenchyma, suggesting that they can both interact with the cells constituting the blood-brain barrier (BBB) and navigate across the BBB via yet-to-be-identified mechanisms. Human exposure to air pollution is associated with endothelial cell damage in the cerebral vasculature, with increases in both intercellular and vascular cell adhesion molecule.<sup>26</sup> Moreover, *in vitro* studies show that exposure to particulates induces the production of cytokines and reactive oxygen species, which is associated with changes in transporter expression and function and a decrease in the expression of various tight-junction proteins in rat brain capillaries. These animal, human, and cell-culture studies have together shown that air pollution is associated with CNS oxidative stress, neuroinflammation, neuronal damage, enhancement of abnormal filamentous proteins, BBB changes, and cerebrovascular damage which provides linkage pathways via which air pollution can impact the pathology of CNS diseases.<sup>27,28</sup>

Air pollution induces neuroinflammation, oxidative stress, cerebrovascular damage, and neurodegenerative pathology. The constituents of air pollution cross from the periphery to the brain via systemic inflammation and the translocation of microparticles into the brain, where the physical properties of the particles may cause damage. Astroglia, brain capillaries, and microglia in particular respond to the various components of air pollution with chronic activation, inflammation, and oxidative stress. Due to the complex nature of this prevalent environmental toxin, CNS pathology may attributable to the synergistic interaction of multiple pathways and mechanisms, making air pollution a potent and biologically

relevant environmental factor that is a significant inducer of mechanistic injury.<sup>29</sup>

The clinical and laboratory results obtained in this study were similar to those of previous studies performed in Turkey. Although the OCB was not investigated in all patients, the value of 60% is lower than the positivity rate of 87.7% found in one of the largest western studies,<sup>30</sup> and it is similar that of 56.1% in an Asian study.<sup>31</sup> These differences could be due to the immunogenetic backgrounds. The female/male ratio in the present study was similar to that in a previous study from Turkey,<sup>5</sup> but not as high as in some European studies. The family history of MS appeared to be high in our study, which could be due to intermarriages among relatives (which are relatively common in Turkey) or the small number of cases.

### Strengths of this study

One of the most important strengths of this study was its design as a door-to-door study with a high participation rate. No hospital records are kept in outpatient hospital clinics in Turkey, except in university and teaching hospitals. The initial diagnosis in outpatients is mostly made based on the immediate clinical findings without any further investigations. In addition to this, outpatients are mostly seen at their next visit in another institution or in private offices, where medical records are unreliable. These factors mean that the results from a prevalence study using hospital records are unlikely to be highly reliable. This study was carried out with assistance from a local neurologist. All but one of the patients were diagnosed by at least two neurologists and at two medical centers; only one patient was diagnosed by us. Another strength was the use of a detailed questionnaire that covered all MS symptoms, and all patients who answered yes to at least one question were examined twice.

### Limitations of the study

This study was subject to several limitations. First of all, the symptoms were reported by the patients themselves, which could have underestimated the prevalence of MS. Another limitation is that other diseases that mimic MS such as neuromyelitis optica spectrum disorder and neurodegenerative diseases can be mistaken for MS. It may be impossible to exclude some of these diseases by applying the McDonald Criteria. Another limitation was that fewer people were screened in Akçakoca.

Furthermore, due to lack of data, other environmental confounding factors that may affect the etiology of MS were not investigated in this study: urbanization, smoking habits, working practices, vitamin D levels, nutrition, vaccinations, viral infections such as the Epstein-Barr virus, cytomegalovirus, and varicella-zoster virus. There may also have been some

other unknown environmental factors that should have been taken into consideration.

In conclusion, the significant difference in the prevalence rate of MS between Karabük and Akçakoca suggests that air pollutants affect the pathogenesis of MS. However, larger epidemiological studies are needed to confirm this hypothesis.

### Conflicts of Interest

The authors have no financial conflicts of interest.

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