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PM10 concentration and microbiological assessment of air in relation to the number of acute cases of type 1 diabetes mellitus in the Lubelskie Voivodeship. Preliminary report

Stężenie pyłu PM10 oraz ocena mikrobiologiczna powietrza atmosferycznego a liczba świeżych zachorowań na cukrzycę typu 1 w województwie lubelskim. Doniesienie wstępne

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Abstract

Introduction. The aim of our study was to evaluate the relation between the concentration of particulate matter of less than 10 μm in diameter (PM10) in air and the effect of psychrophilic bacteria, mesophilic bacteria and mould fungi on the number of new cases of type 1 diabetes mellitus in children and adolescents in the Lubelskie Voivodeship in the years 2015-2016. **Patients and methods.** Epidemiological data on the number of new cases of T1DM was obtained from the Department of Paediatric Endocrinology and Diabetology of the Medical University in Lublin. The number of births for the year 2015 and 2016 in the Lublin Voivodeship was acquired from the statistical yearbook by the Polish Central Statistical Office (GUS). Data on PM10 concentration in the Lubelskie Voivodeship was obtained from the report and annual evaluations of air quality prepared by the Voivodeship Inspectorate of Environmental Protection (WIOŚ) in Lublin. The analysis of psychrophilic bacteria, mesophilic bacteria and mould fungi in air was performed with use of the impact method and an air sampler. **Results.** In the years 2015-2016 in the Lubelskie Voivodeship the number of births was 39 381 and 152 new cases of type 1 diabetes mellitus were recorded. The annual and 24-hour concentration of PM10 in air in 2015 was higher compared to 2016; however, the difference was not statistically significant. Moreover, we detected a higher number of psychrophilic bacteria 2739 vs 1000 CFU/m^3 and a significantly higher number of mesophilic bacteria 92493 vs 1000 CFU/m^3 than the norm specified in the Polish standard PN-89/Z-04111/02. A further analysis of air samples collected in the Lubelskie Voivodeship revealed lower a concentration of mould fungi compared to the Polish standard PN-89/Z-04111/02 (3840 vs 5000 CFU/m^3, respectively). We isolated 9 types of mould fungi and 1 type of yeast-like fungus that are thought to have a negative effect on people's health. The statistical analysis revealed a relation between the number of new cases of T1DM and the number of psychrophilic bacteria ($\beta = 2.86$; $p<0.05$), mesophilic bacteria ($\beta = 2.824$; $p<0.05$) and the number of mould fungi ($\beta=2.923$; $p<0.001$). The analysis of linear regression revealed a relation between the number of new T1DM cases and mean annual concentration of PM10 for the year 2016 ($p<0.001$). However, there was no relation observed between the number of new cases of T1DM and the mean annual concentration of PM10 in air in the Lubelskie Voivodeship in 2015. **Conclusions.** Our preliminary results confirm the not yet fully explored relation between air pollution and the risk of type 1 DM in children and adolescents.

Key words

type 1 diabetes mellitus incidence, children and adolescents, Lubelskie Voivodeship, psychrophilic bacteria, mesophilic bacteria, mould fungi, PM10, air.

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Streszczenie

Wstęp. Celem naszych badań było określenie zależności pomiędzy stężeniem zawieszonego pyłu o średnicy do 10 μm (PM10) w powietrzu atmosferycznym oraz wpływu obecności bakterii psychrofilnych, mezofilnych, grzybów pleśniowych na liczbę świeżych zachorowań na cukrzycę typu 1 u dzieci i młodzieży w województwie lubelskim w latach 2015–2016. **Pacjenci i metody.** Dane epidemiologiczne dotyczące liczby nowych zachorowań na cukrzycę T1DM otrzymano z Kliniki Endokrynologii i Diabetologii Dziecięcej UM w Lublinie. Liczbę urodzeń w latach 2015 i 2016 w województwie lubelskim odczytano z roczników statystycznych GUS. Dane dotyczące stężenia pyłu PM10 w województwie lubelskim uzyskano z raportu i rocznych ocen jakości powietrza, opracowanych przez WIOŚ w Lublinie. Badanie bakterii psychrofilnych, mezofilnych oraz grzybów pleśniowych w powietrzu atmosferycznym wykonano metodą szczelinowo-zderzeniową za pomocą próbnika do poboru powietrza. **Wyniki.** W latach 2015–2016 w województwie lubelskim liczba urodzeń dzieci wynosiła 39 381, a odnotowano 221 nowych przypadków zachorowań na cukrzycę typu 1. W powietrzu atmosferycznym wykazano wyższe w 2015 roku, lecz nie nieistotnie statystycznie roczne jak też 24h stężenie pyłów PM10 w porównaniu do roku 2016. Ponadto wykryto wyższą liczbę bakterii psychrofilnych 2739 vs 1000 CFU/m^3 oraz istotnie wyższą liczbę bakterii mezofilnych 2493 vs 1000 CFU /m^3 w odniesieniu do normy PN-89/Z-04111/02. W dalszej analizie z pobranych próbek powietrza w województwie lubelskim wykryto niższe w porównaniu z normą PN-89/Z-04111/02 stężenie grzybów pleśniowych odpowiednio 3840 vs 5000 CFU/m^3. Jednakże wyizolowano dziewięć gatunków grzybów pleśniowych oraz jeden gatunek drożdżaka o potencjalnym negatywnym wpływie na zdrowie człowieka. W analizie statystycznej wykazano zależność pomiędzy liczbą nowych zachorowań na T1DM a liczbą bakterii psychrofilnych (β= 2,86; $p<0,05$), mezofilnych (β = 2,824; $p<0,05$) oraz liczbą grzybów pleśniowych (β=2,923; $p<0,001$). Analiza regresji liniowej wykazała zależność pomiędzy liczbą nowych zachorowań na T1DM a średnim rocznym stężeniem pyłu PM10 w roku 2016 ($p<0,001$). Natomiast nie wykazano zależności pomiędzy liczbą nowych zachorowań na T1DM a średnim rocznym stężeniem PM10 w powietrzu atmosferycznym w województwie lubelskim w roku 2015. **Wnioski.** Nasze wstępne wyniki badań wspierają słabo poznany związek zanieczyszczenia powietrza a ryzyka rozwoju cukrzycy typu 1 u dzieci i młodzieży.

Słowa kluczowe

zachorowalność na cukrzycę typu1, dzieci i młodzież, woj. lubelskie, bakterie psychrofilne mezofilne, grzyby pleśniowe, pył PM10, powietrze atmosferyczne

Introduction

According to the World Health Organization (WHO) the number of people with diabetes rose from 108 million in 1980 to 422 million in 2014, while the global prevalence of diabetes (standardized according to age) among adults over 18 years of age increased from 4.7% in 1980 to 8.5% in 2014 [1]. In Poland there are approximately 205 000 T1DM patients, out of whom 18 000 are children. Within last 25 years the incidence of T1DM in Poland increased by four times. The highest increase in the T1DM incidence rate is observed among the youngest children aged 0-9 years [2,3]. The rising trend in T1DM incidence both in Poland and in other countries results from environmental factors that lead to the clinical manifestation of this disease in people genetically predisposed to it [2–4]. Studies conducted so far indicate that chemical factors, dyes, preservatives and improvers added to food and stored in plastic containers with bisphenol A (BPA) are the key factors in the etiopathogenesis of diabetes [2,5]. The authors of the most recent study carried out on a diabetic mouse model (C57BL/6 mice) showed a potential diabetogenic relation between bisphenol A and the development of diabetes [6]. Within the last several dozen years, there have been several attempts in the world and Polish literature to assess the general effect of viral infections on the development of type 1 diabetes mellitus [4,5]. It is known from the current studies that even though susceptibility to type 1 diabetes mellitus can be innate, it is the environmental factors, including air pollution, that can trigger the autoagression process and cause the development of the disease [7–10]. Due to the combustion of solid fuels in low power domestic installations not equipped with the flue gas cleaning system, gas emission significantly contributes to the occurrence of episodes of excessive concentrations of PM10 and PM2.5 in air. High prices of solid fuels for individual consumers contribute to the fact that at very low temperatures of air outside, people at their households use waste, including plastic. Consequently, large quantities of organic pollutants of carcinogenic and mutagenic properties are emitted to air. The mutagenic effect of dust pollutants of air is mainly associated with the fact that they contain polycyclic aromatic hydrocarbons, which was confirmed in studies carried out in Wrocław, Kraków and Górny Śląsk [11, 12].

Population studies on the health effects of air pollution are complex. The health effects depend not only on the content of toxic substances in air or the intensity and time of exposure to them, but also on many other individual factors such as place of birth, age, individual resistance, lifestyle and climatic conditions. It is also extremely important to select appropriate statistical models that will allow one to estimate individual exposition to air pollution. New measurement methodologies that allow for the calculation of concentrations and the chemical composition of small dust fractions, and numerous epidemiological studies indicate that the effect of air pollution on people's health is much more severe than it was believed in the 20th century.

In recent years, both in Poland and other countries, environmental pollution has been continuously increasing [7,11,12]. According to the data by the Polish Central Statistical Office

(GUS) for the year 2015, the emission of industrial air pollutants by particularly environmentally unfriendly industrial plants significantly contributed to the fact that the Lubelskie Voivodeship is currently on 10th place on the Polish list of dust emission and 12th place in respect of gas emission. Due to the acceptable 24-hour levels of PM10 concentrations in years 2015 and 2016 having been exceeded, the area of the Lubelskie Voivodeship (Lublin and the area around the city) was classified as class C as regards the level of air pollution [13–15].

Aim of the study

The aim of our study was to evaluate the relation between the concentration of particulate matter of less than 10 μm in diameter (PM10) in air and the effect of psychrophilic bacteria, mesophilic bacteria and mould fungi on the number of acute cases of type 1 diabetes mellitus in children and adolescents in the Lubelskie Voivodeship in the years 2015–2016.

Materials and methods

Samples of air in the Lubelskie Voivodeship were collected in the years 2015–2016. A total number of 27 samples of air was collected. Air samples were collected with use of the impact method with a SAS Super ISO 100 (Italy) sampler which automatically collected 50 or 100 litres of air, depending on the predicted level of pollution. The sucked air was then transported through small holes to a head with a Petri dish containing agar medium appropriate for each type of microorganisms. Samples were collected 1.5 m above the ground. The nozzle of the sampler was positioned perpendicularly to the wind all the time. The mesophilic bacteria were counted after a 24–48-hour incubation at 37°C on TSA by Merck (Germany). In order to culture psychrophilic bacteria we used tryptone soya agar (TSA). Incubation was carried out at 22°C for 72 hours. To culture mould fungi we used the YGC medium by Merck. The plates with growth medium were incubated at 28°C for 5 days.

The numbers of colonies of bacteria and fungi were expressed as a colony-forming unit (CFU) per 1 m^3 of air. When applying the impact method we used the Feller table attached to the manual of the air sampler. Moreover, mould fungi were identified based on macro-and microscopic features, with use of a Nikon Eclipse E2000 microscope and a key for identification of fungi [16]. During sample collection we measured the air temperature humidity and wind direction with a thermo hygrometer by Beurer (Germany).

Epidemiological data

Epidemiological data on the number of new cases of T1DM were obtained from the Department of Paediatric Endocrinology and Diabetology of the Medical University in Lublin. The number of births for the year 2015 and 2016 in the Lublin Voivodeship was acquired from the statistical yearbook by the Polish Central Statistical Office (GUS). Data on the concentration of PM10 μg/m^3 in the Lubelskie Voivodeship was obtained from the report and annual evaluations of air quality prepared by the Voivodeship Inspectorate of Environmental Protection (WIOŚ) in Lublin [13–15].

Assessment of the level of microbiological pollution of air

The assessment of the level of microbiological pollution of air in the Lubelskie Voivodeship was performed based on the norms in the following Polish standards: PN-89/Z-04111/02 and PN-89/Z-04111/02 [17,18].

Statistical analysis

The statistical analysis of the results was performed with use of the R software: (A language and environment for statistical computing) version of 2015 [19]. The degree of intercorrelation between two investigated parameters was assessed with use of the model of linear regression (Poisson distribution). Statistical significance of differences between the groups was set at $p<0.05$.

Results

Number of new cases of T1DM in the Lubelskie Voivodeship in the years 2015–2016

In the years 2015–2016, in Lubelskie Voivodeship, the number of births amounted to 39 381 and there were 152 new cases of T1DM recorded. In the Lubelskie Voivodeship the highest number of new cases of T1DM was recorded in the Lublin County (46 cases) and the lowest number in the Parczew and Janów [prawdopodobnie chodzi o Janów Lubelski – przyp. kor.] County (2 cases). The number of new cases of T1DM per 100 births was the highest in the Hrubieszów County (0.70) and the lowest in the Biała Podlaska County (0.13).

Microbiological assessment of air in the Lubelskie Voivodeship in the years 2015–2016

The microbiological analysis of air in the Lubelskie Voivodeship in the years 2015–2016 showed a higher number of psychrophilic bacteria (2739 vs 1000) CFU /m^3 compared to the norm in the Polish standard PN-89/Z-04111/02 and a significantly higher number of mesophilic bacteria (2493 vs 1000) CFU/m^3 compared to the norm. Moreover, we also observed a lower concentration of mould fungi (3840 vs 5000) CFU /m^3 in the Lubelskie Voivodeship in years 2015–2016, compared to the norm in the Polish standard PN-89/Z-04111/02 [tables I–III].

Qualitative assessment of mould fungi in air in the Lubelskie Voivodeship in years 2015–2016

From the air samples collected in the Lubelskie Voivodeship we isolate 9 types of mould fungi and 1 yeast-like fungus *(Rhodotorula mucilaginosa)* (14.8%). The most commonly isolated types of mould fungi included *Penicillium chrysogenum* (31.7%), *Aspergillus niger* (20.7%) and *Penicillium viridicatum* (7.7%). Less common were *Alternaria alternata* (6.3%), *Paecilomyces sp.* (6.3%), *Aspergillus flavus* (6.0%), *Chrysosporium sp.*

Table I. Number of psychrophilic bacteria in air in the Lubelskie Voivodeship
Tabela I. *Liczba bakterii psychrofilnych w powietrzu atmosferycznym w województwie lubelskim*

Lubelskie Voivodeship Województwo lubelskie	Number of psychrophilic bacteria (CFU/1m^3) Liczba bakterii psychrofilnych (jtk/1m^3)	Polish standard PN-89/Z-04111/02 (CFU /1m^3) Polska norma PN-89/Z-04111/02 (jtk/1m^3)	statistical significance znamienność statystyczna
	2739	1000	$p<0.005$

Table II. Number of mesophilic bacteria in air in the Lubelskie Voivodeship
Tabela II. *Liczba bakterii mezofilnych w powietrzu atmosferycznym w województwie lubelskim*

Lubelskie Voivodeship Województwo lubelskie	Number of mesophilic bacteria (CFU /1m^3) Liczba bakterii mezofilnych (jtk/1m^3)	Polish standard PN-89/Z-04111/02 (CFU /1m^3) Polska norma PN-89/Z-04111/02 (jtk/1m^3)	statistical significance znamienność statystyczna
	2493	1000	$p<0.05$

Table III. Liczba grzybów pleśniowych w powietrzu atmosferycznym w województwie lubelskim
Tabela III*. Number of mould fungi in air in the Lubelskie Voivodeship*

Lubelskie Voivodeship Województwo lubelskie	Number of mould fungi (CFU /1m^3) Liczba grzybów pleśniowych (jtk/1m^3)	Polish standard PN-89/Z-04111/02 (CFU /1m^3) Polska norma PN-89/Z-04111/02 (jtk/1m^3)	statistical significance znamienność statystyczna
	3840	5000	$p<0.05$

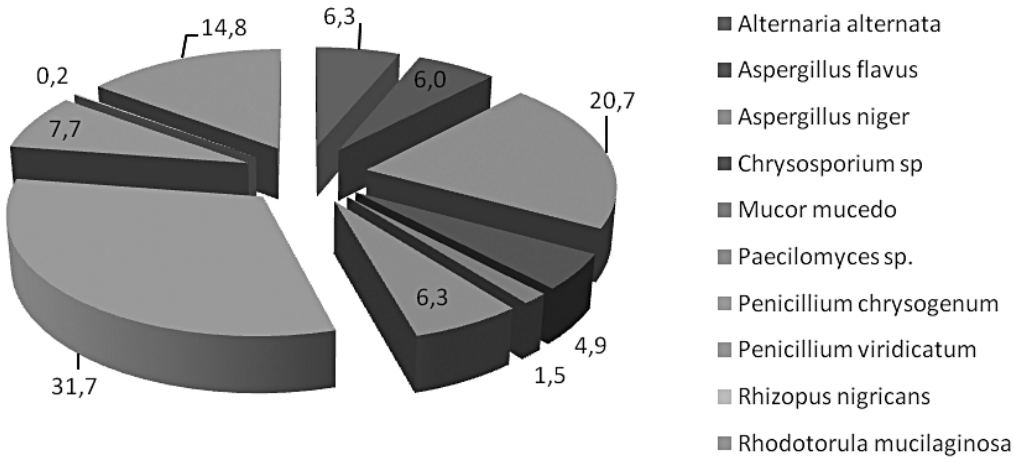

Fig. 1. Percentage of mould and yeast-like fungi isolated from air samples collected in the Lubelskie Voivodeship
Ryc. 1. *Udział procentowy grzybów pleśniowych oraz drożdżaków wyizolowanych z próbek powietrza w województwie lubelskim*

(4.9%) and *Mucor mucedo* (1.5%). The least commonly occurring fungus was *Rhizopus nigricans* (0.2%). The results are presented in fig. 1.

Concentration of PM10 in air in the Lubelskie Voivodeship in years 2015–2016

In 2015, in the Lubelskie Voivodeship, we observed higher annual and 24-hour concentration of PM10 in air compared to year 2016; however, the difference was statistically insignificant (tab. IV).

Linear regression

There was a relation observed in the Pomeranian Voivodeship between the number of new cases of T1DM and the number of psychrophilic bacteria ($\beta = 2.86$; $p<0.05$), mesophilic bacteria ($\beta = 2.824$; $p<0.05$) and mould fungi ($\beta=2.923$; $p<0.001$). Moreover, the analysis of linear regression revealed a relation between the number of new T1DM cases and mean annual concentration of PM10 for the year 2016 ($p<0.001$). However, there was no relation observed between the number of new cases of T1DM and the mean annual concentration of PM10 in air in the Lubelskie Voivodeship in 2015 (tab. V).

Discussion

In the face of the increasing number of new cases of type 1 diabetes mellitus in younger and younger children, researchers

Table IV. Mean annual and 24-hour concentration of PM10 in air in the Lubelskie Voivodeship in the years 2015–2016
Tabela IV. *Średnie roczne oraz 24h stężenie pyłów PM 10 w powietrzu atmosferycznym w województwie lubelskim w latach 2015–2016*

Concentration of PM 10 μg/m3 in air in the Lubelskie Voivodeship Stężenie pyłów PM 10 μg/m^3 w powietrzu atmosferycznym w woj. lubelskim		
Year 2015 Rok 2015	Annual concentration Roczne stężenie	24-hour concentration 24 godzinne stężenie
	32.6	152.0
Year 2016 Rok 2016	28.5	124.4
Statistical significance Znamienność statystyczna	ns	ns

Table V. Relation between the number of new T1DM cases and the number of microorganisms and mean annual PM10 concentration in air – Lubelskie Voivodeship, years 2015–2016
Tabela V. *Zależność pomiędzy liczbą nowych zachorowań na T1DM a liczbą mikroorganizmów oraz średnim rocznym stężeniem pyłu PM10 w badanym powietrzu w województwie lubelskim w latach 2015–2016*

Parameter	β	Statistical significance
Mean annual PM10 concentration vs the number of new T1DM cases in 2015 Średnie roczne stężenie pyłu PM 10 a ilość nowych zachorowań na T1DM w 2015	6.083	ns
Mean annual PM10 concentration vs the number of new T1DM cases in 2016 Średnie roczne stężenie pyłu PM 10 a ilość nowych zachorowań na T1DM w 2016	11.181	<0.001
Psychrophilic bacteria vs the number of new cases of T1DM Bakterie psychrofilne a ilość nowych zachorowań na T1DM	2.863	<0.001
Mesophilic bacteria vs the number of new cases of T1DM Bakterie mezofilne a ilość nowych zachorowań na T1DM	2.824	<0.001
Mould fungi vs the number of new cases of T1DM Grzyby pleśniowe a ilość nowych zachorowań na T1DM	2.923	<0.001

are constantly looking for factors that damage the pancreatic β-cells. In the 20th century environmental factors have been investigated also by other authors from various countries [2,7,8]. According to the literature, the problem of air pollution is most severe in big-city areas [20–22]. Spores of mould fungi or bacteria suspended in air can not only be a direct cause of allergic alveolitis and asthma, but also an etiological factor of many other diseases, including pneumonia, bronchitis and neoplastic diseases [23–25]. In the current microbiological study of air in the Lubelskie Voivodeship in the years 2015–2016 we recorded a higher number of psychrophilic and mesophilic bacteria, compared to the norm. Also, we recorded a lower concentration of yeast-like fungi compared to the Polish standard. However, during further analysis from air samples collected in the Lubelskie Voivodeship we isolated 9 types of mould fungi out of which the most commonly isolated were *Penicillium chrysogenum* (31.7%), *Aspergillus niger* (20.7%), *Penicillium viridicatum* (7.7%), *Alternaria alternata* (6.3%) and *Aspergillus flavus* (6.0%). Moreover, a statistical analysis revealed a relation between the number of mould fungi, psychrophilic and mesophilic bacteria, and the number of new cases of T1DM in the Lubelskie Voivodeship in the years 2015–2016. Studies conducted so far indicate that mould fungi, including *Aspergillus flavus, Aspergillus niger* and *Alternaria alternata,* have a negative effect not only on immunocompromised people but also hematological patients and patients diagnosed with diabetes [24–26]. Tashiro et al. described four cases of patients who fulfilled the criteria of chronic necrotizing pulmonary aspergillosis (CNPA) caused by *Aspergillus niger* and *Aspergillus fumigatus*. One of the patients with diagnosed pulmonary aspergillosis also suffered from diabetes. During the hospitalization the patient was not responding to the mycological treatment and died after two weeks [26]. In other studies authors discussed the effect of PM10 on type 1 diabetes mellitus in children [7, 8, 27]. Di Ciula et al. analysed data from the years 1990–2010 from 16 European countries and collated them with the prevalence of T1DM in children in these countries. The researchers observed that T1DM incidence increased in children with increasing air pollution with PM10 [7]. Hathout et al. studied cases of T1DM children and observed a relation between T1DM and the concentration of PM, in particular in those below the age of 5 years [8]. A similar conclusion was made by Beyerlein et al. in a study conducted in Bavaria on 671 children with a diagnosis of type 1 diabetes. According to the researchers, small particles of PM10, nitrogen dioxide and most probably also PM2.5, generated by road traffic can be a specific factor contributing to the development of T1DM in children below the age of five years [27].

In the area of the Lubelskie Voivodeship the most significant sources of emission of air pollutants include road traffic, agriculture and individual heating of buildings (superficial emission) [13]. Due to the combustion of solid fuels in low power domestic installations not equipped with the flue gas cleaning system, gas emission significantly contributes to the occurrence of episodes of excessive concentrations of PM10 in air. Moreover, both in Poland and other countries, the combustion of domestic waste is a major source of dioxins and furans being released into the air [11,12,28].

In our study we showed a higher annual and 24-hour concentration of PM10 in air in Lubelskie Voivodeship in 2015 compared to 2016. The analysis of linear regression revealed a relation between the number of new T1DM cases and mean annual concentration of PM10 in the year 2016 but not in 2015. It is known that the process of autoimmunization is slow and this is why the time break between the exposure to the risk factor and the onset of the symptoms of diabetes is so long. Despite the fact that many studies have already been conducted in this area, the mechanism that leads to the damage of pancreatic islets β has not yet been fully elucidated [10,29–31]. The identification of the environmental factors that increase the risk of type 1 diabetes mellitus will allow for better understanding of the etiopathogenesis of this disease and a more effective prevention.

Conclusions

Our preliminary results indicate that there is a relation between the number of new cases of T1DM and the number of psychrophilic bacteria, mesophilic bacteria and mould fungi. Moreover, the analysis of linear regression revealed a relation between the number of new cases of T1DM and mean annual concentration of PM10 in the Lubelskie Voivodeship in 2016, but not in 2015. Our preliminary results confirm the not yet fully explored relation between air pollution and the risk of type 1 DM in children and adolescents. It is necessary to carry out further studies in other voivodeships, analysing the effect of environmental pollution on the development of T1DM in children and adolescents.

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Conflict of interest
The author has not provided any conflict of interest.

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