Original Article

Prevalence of pre-clinical autoimmunity in the normal adult population residing in a metropolitan city of India: A cross-sectional study

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Abstract

Objective: A steep rise in the incidence of autoimmune diseases over the decades has been observed, which is simply not explainable with population-level genetic changes, indicating thereby that environmental factors play an important role in their causation. Then again, how air pollution affects the immune system is still not completely elucidated. This study intends to check the presence of autoantibodies and inflammatory markers in normal adults residing for more than 10 years in a highly polluted region.

Methods: In this observational cross-sectional study design, 1,500 subjects residing in Delhi, India, for more than 10 years were screened, of whom 500 were recruited for the study. Distance from the main road to an individual's house was calculated, which was taken as a proxy for traffic pollution exposure. These subjects were analyzed for autoantibodies and inflammatory markers.

Results: The mean age of our cohort was 31.0±8.3 years. Autoantibody positivity was observed in 18% of the subjects, whereas inflammatory markers were elevated in 68% of the subjects. Subjects residing within 200 m of the main road had a higher prevalence of autoantibodies antinuclear antibody (12.5% vs 6.5% p=0.03) and rheumatoid factor (9.6% vs 4.5% p=0.02) than the subjects residing at or more than 250 m away from the main road.

Conclusion: A total of 18% of normal subjects had autoantibody positivity. The odds of developing antinuclear antibodies were twice higher in subjects who resided within 200 m of the main road where the exposure to pollution was higher.

Keywords: Antinuclear antibodies, autoimmunity, cytokine, air pollution, rheumatoid factor, inflammation

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Introduction

In the past decade, the incidence of autoimmune diseases has risen tremendously owing not only to the genetic predisposition but also to several other factors in play. Autoimmune diseases may be triggered or exacerbated by infections, stress, and various other environmental contributors (1, 2). Environmental factors, especially air pollution, affect the immunological health of individuals who are genetically predisposed to develop autoimmune diseases. The air we breathe contains mixture of gases (carbon monoxide, sulfur oxides [sulfur dioxide, SO_2], nitrogen dioxide [NO_2], ozone [O_3], toxic by-products of tobacco smoke, and particulate matter [PM]). PM is composed of both solid and liquid particles (3). Many of the harmful effects on human health caused by air pollutants have been linked to PM smaller than 10 μm in diameter. These particles mainly originate from a large number of automotive vehicles and consequently increase emission in the urban areas. $PM_{2.5}$ are fine particles with particle size of diameter smaller than 2.5 μm, whereas the ultrafine particles have a diameter lesser than 0.1 μm (4). Fine particles in air pollution go unfiltered in the respiratory tract and incite inflammation, which may lead to the production of cytokines, interleukins (ILs), and autoantibodies. This study shall provide knowledge on prevalence of pre-clinical autoimmunity and inflammation in apparently healthy individuals in response to chronic exposure to air pollution.

Methods

Study design
In this observational cross-sectional study, 1,500 subjects were screened on the basis of history and examination. About 500 apparently healthy asymptomatic normal individuals, residing in Delhi, India, for more

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than 10 years, were recruited to participate in the study. Individuals with diabetes, hypertension, obesity, and renal dysfunction; smokers (active or passive); and individuals with family history of autoimmune disease were excluded from the study. Baseline information regarding years of stay at a particular location, occupation, duration of sun exposure/outdoor, distance of the house from the main road (ring road/main branch road), and past medical illness was recorded on a pre-designed proforma. Socioeconomic status was evaluated using the modified Kuppuswamy scale (5). The subjects were requested to send their location by the Global Positioning System. Distance from the main road to each individual's house was calculated using the Google Maps, which was taken as a proxy for traffic pollution exposure (Figure 1). The study was conducted from May 2014 to December 2017. The Ethics Committee of All India Institute of Medical Sciences approved the study (Approval Date: February 04, 2013; Approval Number: IEC/NP-38/2013 & RP-15/04.02.2013). A total of 15 mL of blood was collected from the subjects after obtaining written informed consent. Blood was centrifuged at 2,000 rpm for 10 minutes, and supernatant was collected and immediately stored at -70°C. Samples were tested for pre-decided autoantibodies and inflammatory markers.

Autoantibody and cytokine estimation

Latex agglutination was used for rheumatoid factor (RF) analysis, using Reactivos GPL CHEMELEX, S.A (Barcelona, Spain). The RF concentration was calculated using the following formula: $8 \times RF$ titer=IU/mL. Indirect immunofluorescence method was used for the qualitative determination of antinuclear antibodies (ANA) (antinuclear IgG antibodies) using Hep2 cells. The dilution 1:100 was taken as positive. Sandwich enzyme-linked immunosorbent assay was carried out for checking inflammatory and cytokine markers and the kits used were as follows: IL-17 (D1700), IL-1β/IL-1F2 (DLB50), and tumor necrosis factor (TNF)-α (DTA00C) from R&D systems Inc. (Minneapolis, Minnesota) and high sensitivity (Hs)-C-reactive protein

(CRP) (CR120C) from Calbiotech Inc (El Cajon, California).

Statistical analysis

The sample size was calculated with prevalence of 25% (6) with a precision of 20% using the following formula: sample size $(n)=Z^2PQ/L^2$ (Z: constant (1.96); P: prevalence; Q: 1-p; L: precision). The n obtained was 300. All statistical analysis was done by GraphPad Prism version 5.0 software (GraphPad; San Diego, California, USA). Age was expressed as mean±standard deviation. Fischer exact test was carried out, and the strength of association was expressed as relative risk (RR). In addition, odds ratio (OR) along with confidence interval (CI) were calculated. A p-value of less than 0.05 was taken to be statistically significant.

Results

A total of 500 normal healthy subjects with mean age 31.0±8.3 years were recruited, of whom 77% were female. Within the study population, autoantibodies were observed in 89 (18%) of 500 subjects. The RF was positive in 7% of the subjects, whereas ANA was observed in 9%. IL-6 levels were raised in 172 (34.4%) subjects and were found to be the most prevalent inflammatory marker (Table 1). As per the Kuppuswamy scale, 90.2% participants belonged to the upper middle-class category, whereas lower middle and lower classes were composed of 4.6% participants each, and the rest were in the upper-class category. With regard to subjects spending hours outside, 63% were not working and therefore stayed home, and their maximum spending time outdoors was

less than 10 hours in a week. A total of 37% subjects spent at least 120 hours outside home per week. It was observed that the subjects residing within 200 m of the main road had higher prevalence of autoantibodies than the subjects residing at or more than 250 m away from the main road (ANA [12.5 % vs 6.5%, p=0.03, RR=1.921, OR=2.053, 95% CI=1.103-3.818] and RF [9.6% vs 4.5%, p=0.03, RR=2.160, OR=2.283, 95% CI=1.109-4.702]) (Figure 2). A total of 68% of healthy subjects had inflammatory markers, with IL-6 being elevated in maximum number of individuals (37.9%). Subjects residing within 200 m of the main road showed higher prevalence of inflammatory markers than the subjects residing at or more than 250 m away from

Table 1. Distribution of autoantibodies and inflammatory markers in study population.

Test	Number of subjects (N=500)	%
ANA	45	9
Anti-CCP	13	2.6
RF	33	6.6
TNF-α	60	12
IL-17A	37	7.4
IL-1β	29	5.8
IL-6	172	34.4
Hs-CRP	42	8.4

ANA: antinuclear antibodies; CCP: citrullinated C protein; RF: rheumatoid factor; TNF: tumor necrosis factor; IL: interleukin; Hs-CRP: high sensitivity C-reactive protein.

Main Points

- A total of 18% of healthy individuals had subclinical autoimmunity. In addition, the prevalence of elevated inflammatory markers was high.

- The odds of developing antinuclear antibodies was twice higher in individuals residing in close proximity to the road.

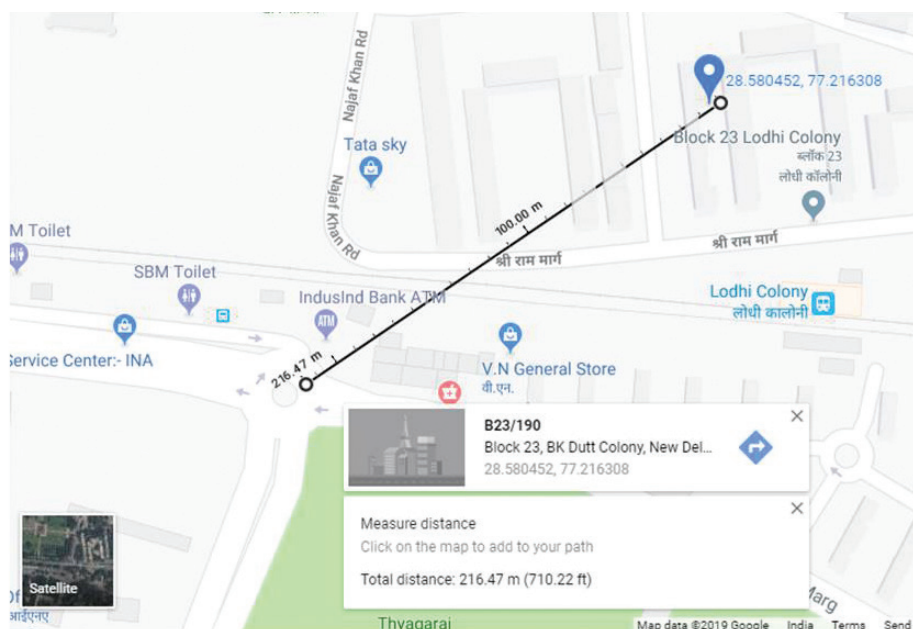- Air pollution remains an important factor in triggering autoimmunity.

Figure 1. Representation of distance measured from the individual's house to the main road/round crossing using Google Maps. This figure shows the distance of the individual's house from the main road/round crossing in meters calculated using the Google Maps.
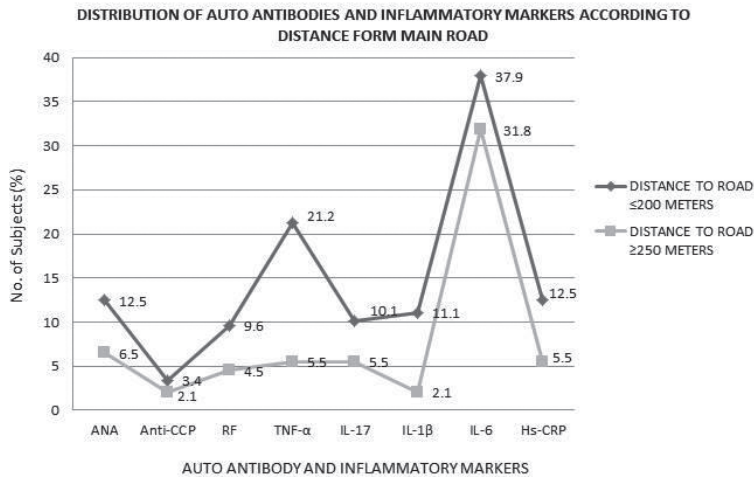
Figure 2. Distribution of autoantibody and inflammatory markers according to distance from the main road.
ANA: antinuclear antibodies; CCP: citrullinated; C protein; RF: rheumatoid factor; TNF: tumor necrosis factor; IL: interleukin; Hs-CRP: high sensitivity; C-reactive protein.

Table 2. Distribution of autoantibodies and inflammatory markers in study population according to the distance from the main road.

Test	Distance to main road ≤200 m (n=208) Number of subjects	Distance to main road ≥250 m (n=292) Number of subjects	OR	95% CI	RR	p
ANA	26	19	2.053	1.10-3.81	1.921	0.026
Anti-CCP	7	6	1.660	0.54-5.015	1.638	0.402
RF	20	13	2.283	1.10-4.70	2.160	0.027
TNF-α	44	16	4.628	2.53-8.46	3.861	0.0001
IL-17	21	16	1.937	0.98-3.81	1.843	0.057
IL-1β	23	6	5.926	2.36-14.83	5.381	0.0001
IL-6	79	93	1.310	0.93-1.52	1.193	0.181
Hs-CRP	26	16	2.464	1.28-4.72	2.281	0.008

p: <0.05.
ANA: antinuclear antibodies; CCP: citrullinated; C protein; RF: rheumatoid factor; TNF: tumor necrosis factor; IL: interleukin; Hs-CRP: high sensitivity; C-reactive protein; OR: odds ratio; CI: confidence interval; RR: relative risk.

the main road (Table 2). There was no subject residing between the distances of 200 and 250 m. The age, gender, and socioeconomic condition of the participants were not found to be significant in univariate and bivariate analysis. Therefore, the adjustment by age, gender, and other variables were not done.

Discussion

With the growing understanding of autoimmune diseases, focus is shifting to prevention studies. Although contribution of genetic influence is <50% in the development of autoimmune disorders, environmental factors may seem to play a major role. Genetically predisposed individuals exposed to PM in the environment tend to be at a higher risk in developing rheumatoid arthritis (RA) and other autoimmune disorders. The inhaled PM induces local lung inflammation, promotion of oxidative stress triggering inflammatory pathways, and reformed immune response leading to autoantibody production, which precedes development of clinical disease by many years. The road density in India was at 1.66 km/km² of area as seen on March 31, 2015—basic road statistics of India 2013-14 and 2014-15. Delhi being the capital of India has the highest road density of 2,103/100 km² than the other states in the country (7).

Several studies have demonstrated association of air pollution and increased RA risk. A 30% increased risk of RA was reported in women living within 50 m of a major road in a large prospective cohort of the Nurses' Health study (8). In addition, exposure to specific air pollutants such as NO_2 and SO_2 were associated with increased risk of RA (9). Autoantibody biomarkers are found to be elevated in patient's sera, 5-10 years before diagnosis or before the actual symptoms of RA appear. Thus, this study is important to spot pre-clinical autoimmunity in the normal adult population residing in a metropolitan city where the air pollution is worse perennially. In our study, the overall prevalence of pre-clinical autoimmunity was found to be 18% with positivity of ANA, RF, and anti-cyclic citrullinated peptide (CCP) being 9%, 6.6%, and 2.6%, respectively, in apparently healthy asymptomatic normal individuals residing in Delhi for more than 10 years. The mean age of the study group (n=500) was 31.0±8.3 years with a female to male ratio of 3.3. As with positive autoantibodies in the observed population, we also witnessed increased levels of inflammatory markers such as TNF-α, IL-17A, IL-1β, IL-6, and Hs-CRP in the population. Individual exposure to traffic pollution was calculated on the basis of the distance of their home from main road/round crossing. About 42% study subjects resided less than 200 m away from the main road, whereas 58% subjects resided more than 250 m away from the main road. We observed a higher prevalence of positive ANA in subjects residing within a distance of 200 m from the main road (12.5%) than in subjects residing at a distance of more than 250 m (6.5%). As a result, the odds of developing ANA will be twice higher in subjects who reside within 200 m from the road. A study from Brazil has reported prevalence of autoantibodies in the serum of 500 normal blood donors to be 22.6% (10). Another study from a rural Canadian population, which aimed at checking pollution owing to herbicide in farmers, reported a positive ANA prevalence of 17.3% at 1:80 titer, where females showed a higher positive percentage than males (20.2% vs 14.9%) (11). ANA was estimated at a dilution 1:100 in this study, and females showed higher positivity. A study from residents' annual physical examination of a small town in Japan over a period of 5 years reported 9.5% positive ANAs at 1:60 titer with higher female prevalence (12). Li et al. (6) reported 25% positive ANA in healthy controls and that the female gender is a possible risk factor for significant ANA positivity. The overall prevalence of ANA conducted in US civilians was found to be 13.8% that in-

creased with age and was higher in females (13). The high positivity of ANA in females as claimed by other studies cannot be truly inferred in this study because of the recruitment of a higher number of female subjects (385) than males (115). In this study, percentage of RF in normal individuals who were residing less than 200 m away from the main road was higher (9.6% vs 4.5%) than people staying more than 250 m away Hence, the risk of developing RF in individuals residing ≤200 m away from the road is 2.3 times that of people residing at a distance ≥250 m. The prevalence of RF positivity as reported from five regions of Cameroon in subjects was found to be 5.6% (14). A cohort study reported 4.3% seropositive RF in healthy controls (15). Another study reported presence of checking RF and anti-CCP antibodyies in reported positivity in 5.7% healthy controls (16). In our study, 2.6% of healthy controls showed positivity for anti-CCP antibodies, and there was not much difference observed in percentage of subjects residing at a distance within or more than 200 m away from the main road/round crossing. Individuals residing within 200 m of the road had a 4.6- and 6-fold higher levels of TNF-α and IL-1β than people residing 250 m away from the main road. Among all the inflammatory markers, IL-6 was observed to be peaked in the studied normal adult population irrespective of the distance to the main road. In addition, individuals residing within 200 m of the road are associated with a more than double increased risk of raised Hs-CRP levels in their serum than individuals residing away. The raised inflammatory markers may possibly be due to higher exposure to the traffic pollution. A retrospective study from Canada found that exposure to ambient levels of O_3 and SO_2 was positively and significantly associated with increased levels of IL-6 (17). A case control study with myocardial infarction reported that long term exposures (over 1, 5 and 30 years) to local traffic-related NO_2 and to residential heating-related SO_2 resulted in a significantly higher CRP and IL-6 levels (18). Another study reported that healthy children with increased exposure to air pollution in Mexico City had their serum levels of inflammatory mediators, including TNF-α, CRP, and IL-1 β, significantly increased when compared with children living in a city with less air pollution. Same authors also reported that children exposed to high levels of $PM_{2.5}$ had significantly increased CRP levels (19, 20). The other studied autoantibodies (anticardiolipin (aCL)-IgG, aCL-IgM, anti–double stranded DNA, anti-liver kidney microsome type 1, anti-neutrophil cytoplasmic antibody) and complements (C_3 and C_4) were found to be negative in the studied normal healthy population. The presence of autoantibodies in normal healthy individuals is not always benign, but it does increase risk for the development of autoimmune rheumatic disease by 10- to 30-folds (21, 22).

The prevalence of the autoimmune and inflammatory markers was high in subjects residing within 200 m of the main road where the exposure to the air pollution was higher. We conclude that air pollution may be one of the important factors in triggering autoimmunity leading to the appearance of autoantibodies in a pre-clinical state and creating an inflammatory state in human body, which may be the nidus for development of autoimmune diseases in individuals. Although further studies are needed to confirm the relevance of autoantibodies in apparently normal individuals as a marker of development of autoimmune rheumatic disease in individuals, it may be possible in future that pre-clinical autoimmunity might one day enable clinicians to identify individuals in the general population who might benefit from risk factor assessment and measures targeted at autoimmune rheumatic disease prevention.

Informed Consent: Informed consent was obtained from the patients who participated in this study.

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Conflict of Interest: The authors have no conflict of interest to declare.

References

1. Adams DD, Knight JG, Ebringer A. Autoimmune diseases: Solution of the environmental, immunological and genetic components with principles for immunotherapy and transplantation. Autoimmun Rev 2010; 9: 525-30. [Crossref]
2. Kivity S, Agmon-Levin N, Blank M, Shoenfeld Y. Infections and autoimmunity - friends or foes?. Trends Immunol 2009; 30: 409-14. [Crossref]
3. Brook RD, Franklin B, Cascio W, Hong Y, Howard G, Lipsett M, et al. Air pollution and cardiovascular disease. Circulation 2004; 109: 2655-71. [Crossref]
4. Pope CA, Dockery DW. Health effects of fine particulate air pollution: Lines that connect. J Air Waste Manage Assoc 2006; 56: 709-42. [Crossref]
5. Bairwa M, Rajput M, Sachdeva S. Modified Kuppuswamy's socioeconomic scale: Social researcher should include updated income criteria, 2012. Indian J Community Med 2013; 38: 185-6. [Crossref]
6. Li Q-Z, Karp DR, Quan J, Branch VK, Zhou J, Lian Y, et al. Risk factors for ANA positivity in healthy persons. Arthritis Res Ther 2011; 13: R38. [Crossref]
7. Government of India, Ministry of Road Transport and Highways, Transport Research Wing. Basic Road Statistics of India 2013-14 and 2014-15. New Delhi; 2016. Available from: https://morth.nic.in/sites/default/files/Basic_Road_Statistics_of_India_2013_14_and_2014_15.pdf
8. Hart JE, Laden F, Puett RC, Costenbader KH, Karlson EW. Exposure to traffic pollution and increased risk of rheumatoid arthritis. Environ Health Perspect 2009; 117: 1065-9. [Crossref]
9. Hart JE, Källberg H, Laden F, Bellander T, Costenbader KH, Holmqvist M, et al. Ambient air pollution exposures and risk of rheumatoid arthritis: Results from the Swedish EIRA case-control study. Ann Rheum Dis 2013; 72: 888-94. [Crossref]
10. Fernandez SAV, Lobo AZC, de Oliveira ZNP, Fukumori LMI, Périgo AM, Rivitti EA. Prevalence of antinuclear autoantibodies in the serum of normal blood donors. Rev Hosp Clin Fac Med Sao Paulo 2003; 58: 315-9. [Crossref]
11. Semchuk KM, Rosenberg AM, McDuffie HH, Cessna AJ, Pahwa P, Irvine DG. Antinuclear antibodies and bromoxynil exposure in a rural sample. J Toxicol Environ Heal Part A 2007; 70: 638-57. [Crossref]
12. Hayashi N, Koshiba M, Nishimura K, Sugiyama D, Nakamura T, Morinobu S, et al. Prevalence of disease-specific antinuclear antibodies in general population: Estimates from annual physical examinations of residents of a small town over a 5-year period. Mod Rheumatol 2008; 18: 153-60. [Crossref]
13. Satoh M, Chan EKL, Ho LA, Rose KM, Parks CG, Cohn RD, et al. Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. Arthritis Rheum 2012; 64: 2319-27. [Crossref]
14. Atabonkeng EP. Evaluation of the prevalence of rheumatoid factor in five regions of Cameroon. Arch Rheumatol 2015; 30: 226-30. [Crossref]
15. Simard JF, Holmqvist M. Rheumatoid factor positivity in the general population. BMJ 2012; 345: e5841. [Crossref]
16. Örge E, Çefle A, Yazıcı A, Gürel-Polat N, Hulagu S. The positivity of rheumatoid factor and anti-cy-

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clic citrullinated peptide antibody in nonarthritic patients with chronic hepatitis c infection. Rheumatol Int 2010; 30: 485-8. [Crossref]

17. Thompson AMS, Zanobetti A, Silverman F, Schwartz J, Coull B, Urch B, et al. Baseline repeated measures from controlled human exposure studies: associations between ambient air pollution exposure and the systemic inflammatory biomarkers IL-6 and fibrinogen. Environ Health Perspect 2010; 118: 120-4. [Crossref]

18. Panasevich S, Leander K, Rosenlund M, Ljungman P, Bellander T, de Faire U, et al. Associations of long- and short-term air pollution exposure with markers of inflammation and coagulation in a population sample. Occup Environ Med 2009; 66: 747-53. [Crossref]

19. Calderón-Garcidueñas L, Villarreal-Calderon R, Valencia-Salazar G, Henríquez-Roldán C, Gutiérrez-Castrellón P, Torres-Jardón R, et al. Systemic inflammation, endothelial dysfunction, and activation in clinically healthy children exposed to air pollutants. Inhal Toxicol 2008; 20: 499-506. [Crossref]

20. Calderón-Garcidueñas L, Macías-Parra M, Hoffmann HJ, Valencia-Salazar G, Henríquez-Roldán C, Osnaya N, et al. Immunotoxicity and environment: Immunodysregulation and systemic inflammation in children. Toxicol Pathol 2009; 37: 161-9. [Crossref]

21. Eriksson C, Kokkonen H, Johansson M, Hallmans G, Wadell G, Rantapää-Dahlqvist S. Autoantibodies predate the onset of systemic lupus erythematosus in northern Sweden. Arthritis Res Ther 2011; 13: R30. [Crossref]

22. Rantapää-Dahlqvist S, de Jong BAW, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003; 48: 2741-9. [Crossref]