

The influence of smoking on multiple sclerosis and autoimmunity

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ABSTRACT. Cigarette smoking is implicated as an environmental risk factor that increases susceptibility to multiple sclerosis (MS) and several other autoimmune diseases. The mechanisms that underlie this effect have not been fully elucidated but they probably are influenced by other environmental exposures and individual genetic background. This review describes evidence that links smoking with autoimmunity with a focus on the associations between smoking and MS risk and disease course.

Key words: smoking, tobacco, multiple sclerosis, autoimmune diseases, risk factors.

RESUMEN. Fumar cigarillos de tabaco es un factor ambiental que aumenta la susceptibilidad a la esclerosis múltiple (EM) y otras enfermedades autoinmunes. Los mecanismos que subyacen a este efecto no han sido completamente elucidados, pero probablemente estén influenciados por otros factores ambientales y el fondo genético individual. Esta revisión describe las evidencias que relacionan el hecho de fumar con la autoinmunidad, con un enfoque especial en la asociación entre el fumar y el riesgo de padecer EM y cómo afecta a su curso.

Palabras clave: fumar, tabaco, esclerosis múltiple, enfermedad autoinmune, factores de riesgo.

Autoimmune diseases are complex disorders that probably result from environmental exposures that influence a predisposed genetic background. For example, exposure to crystalline silica from agricultural or occupational sources, infection with Epstein-Barr virus, and cigarette smoking have all been associated with a number of diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Graves' hyperthyroidism, and primary biliary cirrhosis¹.

Multiple sclerosis (MS) is the most common nontraumatic disabling central nervous system disease of young adulthood and is thought to be autoimmune in origin. Although the target antigen or antigens have not been identified, evidence suggests that MS emerges as a result of complex genetic and environmental interplay with the relevant environmental exposures occurring early in life (probably before age 15 years)². Many individual genes, most with immune system relationships, contribute small independent effects on MS susceptibility and these effects are probably influenced by factors such as systemic infections (e.g., early-life Epstein-Barr virus infection) and ultraviolet light exposure and/or vitamin D status³. Recently, cigarette smoking has joined the list of environmental suspects that may affect MS risk and outcome.

This review will summarize the associations between smoking and autoimmune diseases with a focus on MS. It will also explore the links between smoking and MS disease course, such as influence on measures of disease activity (i.e. clinical relapses

and magnetic resonance imaging (MRI) changes), risk of converting from a relapsing disease course to one of relentless progression, and rate of disability advancement.

□ Why cigarette smoking is relevant to autoimmune disease

Autoimmune disorders may share common genetic mechanisms (for example, association with human leukocyte antigen (HLA) loci) with clinical expression dictated by environmental exposures, their timing and gene-environment interactions. The burden of autoimmune disease appears to be increasing over the past century with increased incidence rates of RA and MS being particularly notable. Moreover, the increasing incidence of MS is shouldered almost entirely by females; the sex ratio has moved from approximately 2:1 (female:male) to 3:1 over the past half-century⁴⁻⁶. It is therefore appealing to consider the roster of social and environmental factors that have changed during that epoch, including urbanization of populations in North America and Europe, the changing role of women in the workplace, changes in timing of menarche and childbirth, and numerous other influences. The use of cigarettes is one of these potential factors. Recently, Palacios and colleagues showed that the sex ratios of MS and smokers are correlated⁷.

Cigarette smoking became popular throughout the world during the 20th century. Global cigarette

Table I**Cigarette smoking: biological effects relevant to immune function**

Mechanism	Specific effects
Tissue injury/apoptosis	- Smoke contains free radicals. - Induction of endogenous free radicals and lipid peroxidation. - Increased cell sensitivity to apoptosis.
Pro-inflammatory	- Increased peripheral leukocytes. - Increased neutrophil chemotaxis and cellular recruitment. - Increased CRP, IL-6 TNF-alpha, and selectins.
Immunosuppression	- Variable effects on T cell function and activation. - Reduced NK cell activity. - Impaired humoral immunity.
Anti-estrogenic	- Formation of inactive 2-hydroxy estrogens.

CRP: C-reactive protein; IL-6: interleukin-6; TNF: tumor necrosis factor. Adapted from Costenbader and Karlson⁸.

consumption increased more than 3-fold between 1950 and 2000 (World Health Organization Tobacco Free Initiative. <http://www.who.int/tobacco/en/atlas8.pdf>). Cigarette smoking prevalence varies geographically and in most regions, more men than women smoke. Some important exceptions where MS rates are high include Norway, Sweden, and New Zealand, all of which have similar cigarette consumption rates for both sexes (World Health Organization Tobacco Free Initiative. <http://www.who.int/tobacco/en/atlas6.pdf>). There exists substantial epidemiologic data regarding smoking in almost every nation and, to some extent, dose and duration of exposure can be quantified for an individual. Moreover, the toxic components of tobacco smoke and its adverse health effects have been subjects of intense study for decades. Therefore, smoking is not only of interest as an environmental influence on risk of autoimmunity but understanding the mechanisms by which it influences risk and disease course could provide therapeutically relevant insights into the underlying biology of MS and other autoimmune diseases.

□ Immunological effects of cigarette smoking

Nicotine is addictive and delivered most effectively as an inhaled aerosol. The burning of tobacco heats the air drawn through a cigarette. As this hot air passes through unburned tobacco, nicotine and other substances evaporate. Subsequent air cooling results in some of the substances condensing into smoke particles. As many as 6000 substances, including aromatic hydrocarbons, tobacco glycoprotein, and metals are released from burning tobacco; many of these substances are known to be antigenic, mutagenic, or carcinogenic^{1, 8}. It appears likely that substances associated with cigarette smoke particles are to blame

for immunological effects and perhaps the increased risk of autoimmunity. Some of this evidence is derived from case-control studies that show that cigarette smoking appears to increase MS risk whereas snuff/moist tobacco use does not (it may actually lower risk). This finding also suggests that nicotine is unlikely to be the sole factor that influences disease risk^{9, 10}. Furthermore, passive exposure to cigarette smoke may also increase MS risk¹¹.

Cigarette smoke has wide-ranging biological effects relevant to immune function (Table I)⁸. It contains high concentrations of free radicals and may activate endogenous sources of free radicals, which in turn may affect DNA, cause gene activation, or direct tissue injury. Furthermore, tobacco smoke contains nitric oxide and cyanide, both of which may have important roles in axonal degeneration and demyelination particularly relevant to MS. Increased lymphocyte apoptosis may result from increased Fas expression and cells such as monocytes and macrophages may release more matrix metalloproteinases. Pro-inflammatory effects include elevation of serum fibrinogen and circulating levels of C-reactive protein and interleukin-6, among others. Certain immune functions may be impaired after cigarette smoke exposure. For example, nicotine results in T cell immunosuppression¹² and cigarette smoke alters B cell and natural killer (NK) cell function¹³⁻¹⁵. Tobacco smoke also has anti-estrogenic effects and female sex hormones may have favorable effects on MS course^{16, 17} as well as on animal models of MS and arthritis.

□ Association of smoking and systemic autoimmune diseases

Numerous independent studies suggest that some of the biological mechanisms described above may in-

tion workers that assessed the relationships between smoking and moist tobacco use with MS and other inflammatory diseases such as rheumatoid arthritis, Crohn's disease, ulcerative colitis, and sarcoidosis⁹. Ever-smoking was associated with an increased risk for all diseases studied except sarcoidosis (lower risk) and the relative risk associated with MS was 1.9 (95% CI 1.4-2.6). In contrast, ever-use of moist snuff, adjusted for smoking, was not associated with any of the diseases (relative risk for MS = 1.0; 95% CI 0.8-1.4). The authors concluded that MS risk is influenced by inhaled non-nicotinic components of cigarette smoke rather than nicotine itself.

Most³⁰⁻³³, though not all³⁴⁻³⁶, recent case-control and cohort studies detected an association of MS susceptibility with smoking. Data from a total of 14 studies were combined in a recently updated meta-analysis³⁷. Data from 3,052 MS patients and 457,619 controls were examined using both a conservative analysis (limited to 10 studies where smoking behavior was described prior to MS onset) and a non-conservative analysis (all 14 studies, regardless of whether smoking behavior was current or occurred prior to MS onset). Smoking was associated with increased MS susceptibility in both models (conservative: risk ratio 1.48, 95% CI 1.35-1.63, $p < 10^{-15}$; non-conservative: risk ratio 1.52, 95% CI 1.39-1.66, $p < 10^{-19}$). Adjustment for median geographic latitude or sex ratio did not alter the results. Of the 14 studies, 11 used a case-control design (meta-analysis relative risk = 1.49 (95% CI 1.35-1.66) whereas three were cohort studies (meta-analysis relative risk = 1.58 (95% CI 1.25-2.00) with similar relative risk estimates ($p = 0.65$).

Some studies included in the meta-analysis suggested a dose-response effect of smoking on MS risk, however, methods of quantifying exposure were not consistent (e.g., some studies evaluated pack-years and others cigarettes per day). A novel study used previously banked blood samples to show that cotinine levels, a measure of recent smoking exposure, were increased in MS patients compared with controls¹¹. Quantitative markers of smoking exposure, especially in a longitudinal prospective cohort, could address the temporality issue of smoking exposure and clinical disease onset and the dose-response relationship between smoking and MS risk.

Cigarette smoking may exert its effect on MS risk at an early stage of life. Although maternal smoking during pregnancy was found not to increase risk in exposed children in one study³⁸ another study found that parental smoking was a risk for subsequent development of MS in offspring³⁹. A recent population-based case-control study demonstrated that MS risk was increased among never-

smokers who had been exposed to passive smoking (OR 1.3, 95% CI 1.1-1.6) compared to never-smokers who had never been exposed⁴⁰. Moreover, MS risk increased with increasing duration of exposure. Because epidemiological data, especially migration studies, suggest that some environmental influences on MS risk probably occur early in life (prior to age 15 years), future research must address the timing of both passive and active cigarette smoke exposure in addition to dose-response effects and interactions with other putative risk factors.

□ Interactions between smoking, other environmental factors and genetics

Environmental risk factors for MS may have independently effects but are likely to interact with other environmental factors and an individual's genetic background, perhaps via several different direct or epigenetic mechanisms^{41, 3}. Examples of environmental risk factors include infection (especially with Epstein-Barr virus) and vitamin D status (or exposure to ultraviolet radiation)⁴². Effects of relative vitamin D deficiency are likely to have impact early in life, Epstein-Barr virus infection probably influences risk during adolescence or early adulthood, primary smoking exposure can occur from late childhood through adulthood, and passive exposure to cigarette smoke can occur in utero and any time thereafter⁴³. Genetic susceptibility is associated with HLA DR15*1501. The influence of each risk factor may be relatively small; only recently have the technology and the large collaborative datasets existed to allow their detection and inferences about the underlying mechanisms. For example, recent studies evaluating the possible role of vitamin D in regulating HLA-DRB1 expression showed that smoking enhanced the association between high anti-Epstein-Barr virus nuclear antigens (EBNA) titer and increased MS risk but did not affect the association between HLA-DR15 and MS risk^{44, 36}. In a population-based Swedish case-control study, there was a significant interaction between two genetic risk factors (presence of HLA-DRB1*15 and absence of HLA-A*02) among smokers but not among non-smokers¹⁰. Compared with non-smokers with neither of the studied genetic risk factors, the odds ratio for MS was 13.5 (95% CI 8.1-22.6) for smokers with both genetic risk factors. The odds ratio for smokers without genetic risk was 1.4 (95% CI 0.9-2.1) and the odds ratio for non-smokers with both genetic risk factors was 4.9 (95% CI 3.6-6.6). Among those with both genetic risk factors, smoking increased MS risk by a factor of 2.8 in those with both genetic risk factors compared with a factor of 1.4 among those

without either genetic risk factor. Despite these results, others have found that smoking may not influence genetically loaded risk within families⁴⁵. Efforts to use data from genome-wide association studies to integrate weighted genetic risk scores for MS susceptibility with environmental factors have emerged⁴⁶. There may be interactions between environmental risk factors as well, for example, current smoking and cumulative tobacco consumption were associated with higher levels of Epstein-Barr virus antibodies⁴⁷.

There are parallels with other autoimmune diseases, especially RA. For example, HLA-DRB1*01 and HLA-DRB1*04 are associated with RA susceptibility. Patients with these epitopes who were also smokers had higher detection rates and titers of autoantibodies recognizing citrullinated proteins⁴⁸. It has been hypothesized that smokers are at particular risk because the biologic effects of cigarette smoke induce mechanisms that citrullinate autoantigens.

A recent study showed that the sex ratios of MS and smokers were correlated, providing not only evidence to support the hypothesis that smoking increases MS risk but a partial explanation for the divergent sex ratio for MS⁷. However, trends in the sex ratio for smoking were primarily determined by reduced rates in men, suggesting that other factors must be sought to explain the increasing incidence of MS in women.

□ Effects of smoking with MS disease course

Once MS has been established, it appears that smoking may be detrimental to the course of the disease. Smoking has been associated with increased disease activity (as measured by clinical relapses and development of new MRI lesions), more rapid conversion from a first demyelinating clinical event (“clinically isolated syndrome” or CIS) to confirmed MS, faster conversion from relapsing-remitting to secondary progressive MS, and faster neurological deterioration once progressive disease takes hold. Evaluation of the magnitude of smoking effect on the course of established MS may shed light on underlying disease mechanisms and is important because more than half of MS patients smoke at some time⁴⁹.

An association between cigarette smoking and acute MS exacerbations was observed in the 1960s⁵⁰. Smoking was also linked to transient deterioration in motor performance of about 10 minutes duration in 21 MS patients compared with 11 healthy controls in whom steady improvement was observed⁵¹. However, there has been little subsequent research on this specific issue.

Three-quarters of patients with a first demyelinating event (CIS) who were also smokers had converted to definite MS after 36 months whereas only 51% of their nonsmoking counterparts had converted during a similar time frame (hazard ratio 1.8 (95% CI, 1.2–2.8) for smokers compared with non-smokers ($p = 0.008$))⁵². Smokers had a significantly shorter time interval to their first relapse; the authors concluded that smoking speeds conversion from CIS to confirmed MS.

Studies that evaluated the association of smoking and the rate of MS progression or disability have shown variable results. A cross-sectional Dutch survey showed no effect of smoking on disease progression or disability⁵³. However, Hernan et al found that smoking increased the risk of conversion from relapsing-remitting to secondary progressive disease (hazard ratio 3.6 (95% CI 1.3-9.9 for ever-smokers compared with never-smokers)³⁰. A Swedish study of self-report data from 122 newly diagnosed MS patients found that after a median of 6 years, ever-smokers were more likely to have progressive disease compared with never-smokers ($p < 0.01$)⁵⁴. Moreover, the effects on the rate of conversion to progressive MS and earlier age of progression onset were greatest in those who began smoking before age 15 years. Early smoking start was also associated with a higher rate of primary progressive MS when compared with never smokers ($p = 0.012$).

These findings were largely replicated by a larger ($n=1465$) study of MS patients studied using a cross-sectional survey and the subsequent prospective follow-up for a mean of 3.29 years⁵⁵. More than half ($n=780$; 53.2%) were never-smokers, 428 (29.2%) were ex-smokers, and 257 (17.5%) were current smokers. Current smokers had significantly worse baseline function than never-smokers in terms of Expanded Disability Status Scale (EDSS) score (adjusted $P < .001$), Multiple Sclerosis Severity Score (MSSS; adjusted $P < .001$), and brain parenchymal fraction (adjusted $P = .004$). In addition, current smokers were significantly more likely to have primary progressive MS (adjusted odds ratio, 2.41; 95% CI, 1.09-5.34). At follow up, progression to secondary progressive disease occurred faster in smokers than in never-smokers (hazard ratio for current smokers versus never-smokers, 2.50; 95% CI, 1.42-4.41). In addition, brain MRI T2-weighted lesion volume increased faster ($P = .02$), and brain parenchymal fraction decreased faster ($P = .02$) in smokers compared with nonsmokers. Another prospective study from Tasmania followed 203 MS patients for a median of 909 days, finding a dose-response effect (measured using cumulative pack-years) of smoking on disability⁵⁶. Greater consumption was associated

with an increase in longitudinal MSSS ($p < 0.001$); similar findings were noted when the EDSS was used as the disability outcome. Smoking during the cohort period was not associated with relapse (cumulative pack years smoked after cohort entry: hazard ratio 0.94 (95% CI 0.69-1.26) per pack year). Smoking was identified as a risk factor for a so-called “sustained malignant” course of MS with progression to need gait aid within 5 years of disease onset⁵⁷.

A meta-analysis of four studies concluded that although there was significant heterogeneity between the studies, there was a trend towards smoking increasing the risk of secondary progressive MS (relative risk 1.88; 95% CI 0.98-3.61; $p = 0.06$)³⁷.

Little is known about the effects of smoking on response to disease-modifying MS therapies. A small study of 31 MS patients using subcutaneous interferon-beta revealed that smokers were found to have a much greater risk of developing neutralizing antibodies that interfere with biological activity of the drug⁵⁸. Smoking status has been associated with reduced treatment responsiveness in RA. Interestingly, smoking was identified as a risk factor for emergence of systemic autoimmunity, especially thyroid disease, in MS patients treated with the anti-CD52 monoclonal antibody alemtuzumab⁵⁹.

□ Summary and future research

Current evidence suggests that cigarette smoking is associated with increased MS susceptibility (relative risk approximately 1.5) and increased risk of other systemic autoimmune diseases. This evidence

only partially fulfills criteria for causation such as specificity, temporality, and biological gradient (dose-response effect), but other criteria such as consistency and biological plausibility are gaining epidemiological and scientific credence. Smoking is also likely associated with alteration of clinical course once disease is established. There is a greater risk of conversion from CIS to MS and from relapsing-remitting to secondary progressive MS in smokers. Disability appears to accumulate faster in smokers who have established progressive forms of MS. The effects of smoking on the response to MS disease-modifying therapies are not known but are of concern because smoking is reported to reduce drug effectiveness in RA.

The mechanisms by which smoking might influence the risk of MS and its clinical course are unclear. Prospective studies that include genetic covariates, account for potential confounders, and accurately quantify smoking exposure are needed to improve our understanding of the magnitude of effect of smoking on MS and other autoimmune diseases and to uncover the mechanisms that underlie those effects. It would be of great interest to contrast the harmful biological effects of smoking on MS and other autoimmune diseases with the apparent protective effect of smoking for Parkinson disease, a neurodegenerative disease with genetic and environmental influences⁶⁰. Insights from each perspective may reveal underlying biological mechanisms of general autoimmunity, tissue injury, and neurodegeneration, leading to new avenues for disease prevention and therapy.

BIBLIOGRAFÍA

- 1.- Arnson Y, Shoenfeld Y, Amital H. Effects of tobacco smoke on immunity, inflammation and autoimmunity. *Journal of Autoimmunity* 2010;34(3): J258-65.
- 2.- Compston A, Coles A. Multiple sclerosis. *Lancet* 2008; 372:1502-17.
- 3.- van der Mei IA, Simpson S, Jr., Stankovich J, Taylor BV, van der Mei IAF. Individual and joint action of environmental factors and risk of MS. *Neurol Clin* 2011;29(2):233-55.
- 4.- Celius EG, Smestad C. Change in sex ratio, disease course and age at diagnosis in Oslo MS patients through seven decades. *Acta Neurol Scand* 2009;Supplementum (189):27-9.
- 5.- Maghzi AH, Ghazavi H, Ahsan M, Etemadifar M, Mousavi S, Khorvash F, et al. Increasing female preponderance of multiple sclerosis in Isfahan, Iran: a population-based study. *Mult Scler* 2010;16(3):359-61.
- 6.- Orton SM, Herrera BM, Yee IM, Valdar W, Ramagopalan SV, Sadovnick AD, et al. Sex ratio of multiple sclerosis in Canada: a longitudinal study. *Lancet neurol* 2006;5(11):932-6.
- 7.- Palacios N, Alonso A, Bronnum-Hansen H, Ascherio A. Smoking and increased risk of multiple sclerosis: parallel trends in the sex ratio reinforce the evidence. *Annals of Epidemiology* 2011;21(7):536-42.
- 8.- Costenbader KH, Karlson EW. Cigarette smoking and autoimmune disease: what can we learn from epidemiology? *Lupus* 2006;15(11):737-45.
- 9.- Carlens C, Hergens MP, Grunewald J, Ekblom A, Eklund A, Hoglund CO, et al. Smoking, use of moist snuff, and risk of chronic inflammatory diseases. *Am J Respir Crit Care Med* 181(11):1217-22.

- 10.- Hedstrom AK, Baarnhielm M, Olsson T, Alfredsson L, Hedstrom AK, Baarnhielm M, et al. Tobacco smoking, but not Swedish snuff use, increases the risk of multiple sclerosis. *Neurology* 2009;73(9):696-701.
- 11.- Sundstrom P, Nystrom L, Hallmans G. Smoke exposure increases the risk for multiple sclerosis. *Eur J Neurol* 2008;15(6):579-83.
- 12.- Sopori ML, Kozak W. Immunomodulatory effects of cigarette smoke. *J Neuroimmunol* 1998;83(1-2):148-56.
- 13.- Fusby JS, Kassmeier MD, Palmer VL, Perry GA, Anderson DK, Hackfort BT, et al. Cigarette smoke-induced effects on bone marrow B-cell subsets and CD4⁺:CD8⁺ T-cell ratios are reversed by smoking cessation: influence of bone mass on immune cell response to and recovery from smoke exposure. *Inhal Toxicol* 2010;22(9):785-96.
- 14.- Motz GT, Eppert BL, Wesselkamper SC, Flury JL, Borchers MT. Chronic cigarette smoke exposure generates pathogenic T cells capable of driving COPD-like disease in Rag2^{-/-} mice. *Am J Respir Crit Care Med* 2010;181(11):1223-33.
- 15.- Motz GT, Eppert BL, Wortham BW, Amos-Kroohs RM, Flury JL, Wesselkamper SC, et al. Chronic cigarette smoke exposure primes NK cell activation in a mouse model of chronic obstructive pulmonary disease. *J Immunol* 2010;184(8):4460-9.
- 16.- Gold SM, Voskuhl RR. Estrogen treatment in multiple sclerosis. *J Neurol Sci* 2009;286(1-2):99-103.
- 17.- Michnovicz JJ, Hershcopf RJ, Naganuma H, Bradlow HL, Fishman J. Increased 2-hydroxylation of estradiol as a possible mechanism for the anti-estrogenic effect of cigarette smoking. *N Engl J Med* 1986;315(21):1305-9.
- 18.- Westhoff G, Rau R, Zink A. Rheumatoid arthritis patients who smoke have a higher need for DMARDs and feel worse, but they do not have more joint damage than non-smokers of the same serological group. *Rheumatology* 2008;47:849-54.
- 19.- Matthey DL, Brownfield A, Dawes PT. Relationship between pack-year history of smoking and response to tumor necrosis factor antagonists in patients with rheumatoid arthritis. *J Rheumatology* 2009;36:1180-7.
- 20.- Sugiyama D, Nishimura K, Tamaki K, Tsuji G, Nakazawa T, Morinobu A, et al. Impact of smoking as a risk factor for developing rheumatoid arthritis: a meta-analysis of observational studies. *Ann Rheum Dis* 2010;69(1):70-81.
- 21.- Costenbader KH, Kim DJ, Peerzada J, Lockman S, Nobles-Knight D, Petri M, et al. Cigarette smoking and the risk of systemic lupus erythematosus: a meta-analysis. *Arthritis Rheum* 2004;50:849-57.
- 22.- Holm IA, Manson JE, Michels KB, Alexander EK, Willett WC, Utiger RD. Smoking and other lifestyle factors and the risk of Graves' hyperthyroidism. *Arch Int Med* 2005;165:1606-11.
- 23.- Stan MN, Bahn RS. Risk factors for development or deterioration of Graves' ophthalmopathy. *Thyroid* 2010;20(7):777-83.
- 24.- Antonovsky A, Leibowitz U, Smith HA, Medalie JM, Balogh M, Kats R, et al. Epidemiologic study of multiple sclerosis in Israel. I. An overall review of methods and findings. *Arch Neurol* 1965;13:183-93.
- 25.- Simpson CA, Newell DJ, Schapira K. Smoking and multiple sclerosis. *Neurology* 1966;16(10):1041-3.
- 26.- Villard-Mackintosh L, Vessey MP. Oral contraceptives and reproductive factors in multiple sclerosis incidence. *Contraception* 1993;47(2):161-8.
- 27.- Thorogood M, Hannaford PC. The influence of oral contraceptives on the risk of multiple sclerosis. *Br J Obstet Gynaecol* 1998;105(12):1296-9.
- 28.- Ghadirian P, Dadgostar B, Azani R, Maisonneuve P. A case-control study of the association between socio-demographic, lifestyle and medical history factors and multiple sclerosis. *Can J Public Health* 2001;92(4):281-5.
- 29.- Hernan MA, Olek MJ, Ascherio A. Cigarette smoking and incidence of multiple sclerosis. *Am J Epidemiol* 2001;154(1):69-74.
- 30.- Hernan MA, Jick SS, Logroscino G, Olek MJ, Ascherio A, Jick H, et al. Cigarette smoking and the progression of multiple sclerosis. *Brain* 2005;128(Pt 6):1461-5.
- 31.- Pekmezovic T, Drulovic J, Milenkovic M, Jarebinski M, Stojisavljevic N, Mesaros S, et al. Lifestyle factors and multiple sclerosis: A case-control study in Belgrade. *Neuroepidemiology* 2006;27(4):212-6.
- 32.- Riise T, Nortvedt MW, Ascherio A, Riise T, Nortvedt MW, Ascherio A. Smoking is a risk factor for multiple sclerosis. *Neurology* 2003;61(8):1122-4.
- 33.- Rodriguez Regal A, del Campo Amigo M, Paz-Esquete J, Martinez Feijoo A, Cebrian E, Suarez Gil P, et al. [A case-control study of the influence of the smoking behaviour in multiple sclerosis]. *Neurologia* 2009;24(3):177-80.
- 34.- Russo C, Morabito F, Luise F, Piriomalli A, Battaglia L, Vinci A, et al. Hyperhomocysteinemia is associated with cognitive impairment in multiple sclerosis. *J Neurol* 2008;255(1):64-9.
- 35.- Silva KR, Alvarenga RM, Fernandez YFO, Alvarenga H, Thuler LC, Silva KRPd, et al. Potential risk factors for multiple sclerosis in Rio de Janeiro: a case-control study. *Arq Neuropsiquiatr* 2009;67(2A):229-34.
- 36.- Simon KC, van der Mei IA, Munger KL, Ponsonby A, Dickinson J, Dwyer T, et al. Combined effects of smoking, anti-EBNA antibodies, and HLA-DRB1*1501 on multiple sclerosis risk. *Neurology* 2010;74(17):1365-71.

- 37.- Handel AE, Williamson AJ, Disanto G, Dobson R, Giovannoni G, Ramagopalan SV, et al. Smoking and multiple sclerosis: an updated meta-analysis. *PLoS ONE* 2011;6(1):e16149.
- 38.- Montgomery SM, Bahmanyar S, Hillert J, Ekbom A, Olsson T. Maternal smoking during pregnancy and multiple sclerosis amongst offspring. *Eur J Neurol* 2008;15(12):1395-9.
- 39.- Mikaeloff Y, Caridade G, Tardieu M, Suissa S, group Ks, Mikaeloff Y, et al. Parental smoking at home and the risk of childhood-onset multiple sclerosis in children. *Brain* 2007;130(Pt 10):2589-95.
- 40.- Hedstrom AK, Baarnhielm M, Olsson T, Alfredsson L. Exposure to environmental tobacco smoke is associated with increased risk for multiple sclerosis. *Mult Scler* 2011;17(7):788-93. doi:10.1177/1352458511399610.
- 41.- Handel AE, Ebers GC, Ramagopalan SV, Handel AE, Ebers GC, Ramagopalan SV. Epigenetics: molecular mechanisms and implications for disease. *Trends in Molecular Medicine* 2009;16(1):7-16.
- 42.- Wingerchuk DM. Environmental factors in multiple sclerosis: Epstein-Barr virus, vitamin D, and cigarette smoking. *Mt Sinai J Med* 2011;78(2):221-30.
- 43.- Handel AE, Giovannoni G, Ebers GC, Ramagopalan SV, Handel AE, Giovannoni G, et al. Environmental factors and their timing in adult-onset multiple sclerosis. *Nature Reviews Neuroscience* 2010;6(3):156-66.
- 44.- Handunnetthi L, Ramagopalan SV, Ebers GC, Handunnetthi L, Ramagopalan SV, Ebers GC. Multiple sclerosis, vitamin D, and HLA-DRB1*15. *Neurology* 2010;74(23):1905-10.
- 45.- Jafari N, Hoppenbrouwers IA, Hop WC, Breteler MM, Hintzen RQ, Jafari N, et al. Cigarette smoking and risk of MS in multiplex families. *Mult Scler* 2009;15(11):1363-7.
- 46.- De Jager PL, Chibnik LB, Cui J, Reischl J, Lehr S, Simon KC, et al. Integration of genetic risk factors into a clinical algorithm for multiple sclerosis susceptibility: a weighted genetic risk score. *Lancet neurol* 2009;8(12):1111-9.
- 47.- Nielsen TR, Pedersen M, Rostgaard K, Frisch M, Hjalgrim H. Correlations between Epstein-Barr virus antibody levels and risk factors for multiple sclerosis in healthy individuals. *Mult Scler* 2007;13(3):420-3.
- 48.- Klareskog L, Malmstrom V, Lundberg K, Padyukov L, Alfredsson L. Smoking, citrullination and genetic variability in the immunopathogenesis of rheumatoid arthritis. *Seminars in Immunology* 2011; 23(2):92-8.
- 49.- Marrie RA, Cutter G, Tyry T, Campagnolo D, Vollmer T, Marrie RA, et al. Smoking status over two years in patients with multiple sclerosis. *Neuroepidemiology* 2009;32(1):72-9.
- 50.- Courville CB, Maschmeyer JE, Delay CP. Effects of smoking on the acute exacerbations of multiple sclerosis. *Bull Los Angel Neuro Soc* 1964;29:1-6.
- 51.- Emre M, de Decker C. Effects of cigarette smoking on motor functions in patients with multiple sclerosis. *Arch Neurol* 1992;49(12):1243-7.
- 52.- Di Pauli F, Reindl M, Ehling R, Schautzer F, Gneiss C, Lutterotti A, et al. Smoking is a risk factor for early conversion to clinically definite multiple sclerosis. *Mult Scler* 2008;14(8):1026-30.
- 53.- Koch M, van Harten A, Uyttenboogaart M, De Keyser J, Koch M, van Harten A, et al. Cigarette smoking and progression in multiple sclerosis. *Neurology* 2007;69(15):1515-20.
- 54.- Sundstrom P, Nystrom L. Smoking worsens the prognosis in multiple sclerosis. *Mult Scler* 2008; 14(8):1031-5.
- 55.- Healy BC, Ali EN, Guttmann CR, Chitnis T, Glanz BI, Buckle G, et al. Smoking and disease progression in multiple sclerosis. *Arch Neurol* 2009;66(7):858-64.
- 56.- Pittas F, Ponsonby AL, van der Mei IA, Taylor BV, Blizzard L, Groom P, et al. Smoking is associated with progressive disease course and increased progression in clinical disability in a prospective cohort of people with multiple sclerosis. *J Neurol* 2009;256(4):577-85.
- 57.- Gholipour T, Healy B, Baruch NF, Weiner HL, Chitnis T. Demographic and clinical characteristics of malignant multiple sclerosis. *Neurology* 2011; 76(23):1996-2001.
- 58.- Sena A, Bendtzen K, Cascais MJ, Pedrosa R, Ferret-Sena V, Campos E, et al. Influence of apolipoprotein E plasma levels and tobacco smoking on the induction of neutralising antibodies to interferon-beta. *J Neurol* 2010;257(10):1703-7.
- 59.- Cossburn M, Pace AA, Jones J, Ali R, Ingram G, Baker K, et al. Autoimmune disease after alemtuzumab treatment for multiple sclerosis in a multicenter cohort. *Neurology* 2011;77(6):573-9.
- 60.- Chen H, Huang X, Guo X, Mailman RB, Park Y, Kamel F, et al. Smoking duration, intensity, and risk of Parkinson disease. *Neurology* 2010;74(11):878-84.