Season of infectious mononucleosis as a risk factor for multiple sclerosis: a UK primary care case-control study.

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Abstract

Background: Infectious mononucleosis (IM) and vitamin D deficiency are both risk factors for multiple sclerosis (MS).

Objective: We wished to establish if IM in the winter months when vitamin D levels are low may be a greater risk factor for MS than IM in the summer months.

Methods: We identified all patients with MS diagnosed aged 16 to 60 in a large primary care database in the United Kingdom and matched each by age, sex, general practice and observation period with up to six controls. We identified a coded diagnosis of IM prior to the index date (date of diagnosis). Logistic regression was used to calculate the odds ratio for prior IM exposure in cases versus controls and for winter versus summer exposure in cases and controls with prior IM exposure.

Results: Based on 9,247 cases and 55,033 matched controls (246 and 846 with prior IM respectively), IM was associated with the development of MS (OR 1.77, 95%CI 1.53-2.05) but there was no evidence that IM in the winter as opposed to summer was associated with developing MS (OR 1.09, 95%CI 0.72-1.66).

Conclusion: We found no evidence that the season of IM influences the risk of subsequent MS.

Keywords: multiple sclerosis, infectious mononucleosis, seasons, risk factors

Introduction

Increasing latitude (Ascherio and Munger, 2007a) and infection with Epstein Barr Virus (EBV) (Ascherio and Munger, 2007b, Almohmeed et al., 2013, Holmøy, 2008) are both known risk factors for multiple sclerosis (MS). The geographical variation in MS is thought to be at least partly due to lower vitamin D levels because of lower sunlight exposure at higher latitudes. This is supported by evidence that lower serum vitamin D levels are associated with a higher risk of developing MS (Munger et al., 2006) although vitamin D replacement has not yet been shown to improve clinical outcomes (James et al., 2013).

Patients with MS are significantly more likely to be sero-positive for anti-EBV antibodies than controls (95% vs 86%), indicating a higher risk of any prior infection with EBV (Almohmeed et al, 2013). They are also more likely to have had symptomatic infectious mononucleosis (glandular fever) due to first exposure to EBV in adolescence rather than childhood (2-14% in MS patients vs 0.7-7% in controls, depending on method of ascertainment) (Handel et al., 2010, Lossius et al., 2010, Marrie et al, 2000). These rates are much lower than the rate of EBV sero-positivity, which is consistent with the fact that most EBV exposure is in early life when it is relatively asymptomatic Individuals with MS also display an unusual immune response to EBV, with significantly higher anti-EBV antibody titres present for many years prior to the onset of symptoms compared to EBV sero-positive controls who do not develop MS (Levin et al., 2005).

Vitamin D has multiple effects on immune function such as increasing the production of the anti-inflammatory cytokine IL-10, inducing T-regulatory cells, and promoting tolerance to self-antigens (Aranow, 2011). IL-10 plays an important role in the immune response to latent EBV infection (Marshall et al., 2003, Marshall et al., 2007). It has been suggested that vitamin D modulates the immune response to EBV (Holmøy, 2008) so that vitamin D deficiency at the time of infection with EBV could increase the risk of subsequently developing MS (Ascherio and Munger, 2007b, Holmøy, 2008). If a vitamin D dependent immune response to EBV is indeed related to the risk of subsequently developing MS, infectious mononucleosis in the winter months when serum vitamin D levels are at their lowest would be hypothesised to be more strongly associated with MS than infectious mononucleosis in the summer. A single case-control study of 1660 MS patients and 3050 controls found no relationship between the risk of MS and season of infectious mononucleosis (Lossius et al., 2014) but this relied on patient self-reporting of infectious mononucleosis and has not been replicated.

We aimed to: (a) confirm whether MS patients in the United Kingdom (UK) are more likely to have had previous infectious mononucleosis than controls; (b) assess whether infectious mononucleosis in winter (defined by either months with lowest recorded vitamin D levels [December-May] (Hypponen and Power, 2007) or lowest sunlight [October-March] (UK Climate Period) is a stronger risk factor for MS than infectious mononucleosis occurring in summer.

Materials and Methods

The Clinical Practice Research Datalink [CPRD] (CPRD homepage) holds routinely-collected, quality-assured, anonymised clinical data from primary care on over 10 million patients in the UK. We requested data for all MS cases aged 16 years and over with a first coded diagnosis of multiple sclerosis or, if no MS code, a first prescription of a MS disease modifying agent from 1990-2010 (see supplementary tables e1 and e2 for codes used). All patients aged 16-60 who were registered on the CPRD for a minimum of two years and had a first coded diagnosis of MS were identified. Cases were matched with up to six control patients by age, gender, general practice and duration of observation in the CPRD prior to the date of MS diagnosis (index date).

We identified the date of onset of MS as either the date of first presentation with a symptom or diagnosis (e.g. optic neuritis) consistent with MS (see supplementary table e3 for codes), or if there were no such symptoms recorded, the date a diagnosis of MS was made.

Patients were defined as having had infectious mononucleosis if they had a coded diagnosis of infectious mononucleosis, or had a “positive” or “abnormal” infectious mononucleosis test result prior to the onset of MS (see supplementary tables e4 and e5 for codes). Patients who had a recorded diagnosis of infectious mononucleosis but who had received a negative infectious mononucleosis test result within seven days of the diagnosis being made were regarded as having not had infectious mononucleosis. The time of year of glandular fever was identified from the date of entry of the infectious mononucleosis diagnosis or positive test result code.

To assess whether MS patients were more likely to have had previous infectious mononucleosis than their matched controls, we calculated an odds ratio (OR) with conditional logistic regression. We then analysed with non-conditional logistic regression only those cases and controls with a recorded date of infectious mononucleosis to assess whether infectious mononucleosis in December to May was associated with an increased risk of MS than infectious mononucleosis occurring during June to November, controlling for age at index date, sex and geographical region. Statistical analysis was performed in StatsDirect v2.5.6. Sample size calculations were based on the rates of infectious mononucleosis in MS cases and controls previously reported in the CPRD database (Marrie et al., 2000). One thousand three hundred MS cases and 7,700 controls would demonstrate that MS is associated with a 2.3 fold increase in the odds of infectious mononucleosis (5% significance level, 80% power), as found in a previous meta-analysis (Thacker et al., 2006). One hundred and sixty MS patients and 160 controls would be required to detect a two-fold increase in the odds of winter infectious mononucleosis in MS patients (5% significance, 80% power). The project was reviewed and accepted by the Independent Scientific Advisory Committee for MHRA database research but did not require ethics approval as the data were all anonymised.

Results

After exclusions (Figure 1), analysis was performed on 9,247 cases and 55,033 matched controls, mean index age 41 years, 71% female. The median observation period in the GPRD was 24.5 years (interquartile range 15.2 to 35.8). In 13% MS patients there was a date of first attack prior to the date of diagnosis (mean age 36 years). The mean ages of infectious mononucleosis was 21.7 (SD 9.3) and 19.4 (SD

7.5) years in patients and controls respectively and the median time from the diagnosis of infectious mononucleosis to the diagnosis of MS was 15.0 years (interquartile range 9.2 to 22.8 years). Prior infectious mononucleosis occurred in 246/9,247 (2.7%) MS patients and 846/55,033 (1.5%) controls and conditional logistic regression showed prior infectious mononucleosis exposure was more common in MS cases than controls (OR 1.77, 95%CI 1.53-2.05).

1,092 patients had a prior diagnosis of infectious mononucleosis of whom 128/246 (52%) cases and 363/846 (43%) controls were excluded because the date of infection was unclear (Table 1). The difference in proportions of excluded data was significant (p=0.013). The case and control groups with prior infectious mononucleosis were similar in terms of age at index date (both 34 years) and sex (75% and 78% female respectively). Exposure to infectious mononucleosis during winter was not associated with a higher risk of MS than exposure during summer months, whether defined by low vitamin D levels (OR 1.09, 95%CI 0.72-1.66) or sunlight (OR 0.96, 95%CI 0.63-1.45). Including only MS patients with a date of first attack did not alter the results, although only 19 MS patients with infectious mononucleosis were included (vitamin D OR 0.61, 95%CI 0.20, 2.34, sunlight OR 0.84, 95%CI 0.30, 2.34).

Table 1: Season of infectious mononucleosis in cases and controls

	MS Cases		**Controls**		**Total**
	Sunlight*	Vit D†	Sunlight*	Vit D†	
Prior IM	246		846		1,092
Winter	60	70	250	270	340
Summer	58	48	233	213	261

Season unclear	128	363	491
No prior IM coded	9,001	54,187	63,188
Total	9,247	55,033	64,280

MS – multiple sclerosis; IM – infectious mononucleosis

* winter = October to March inclusive

† winter = December to May inclusive

Discussion

We confirmed that previous infectious mononucleosis significantly increases the risk of MS, in keeping with a recent meta-analysis (19,390 cases, 16,007 controls from 18 individual studies), which showed a relative risk of MS with prior infectious mononucleosis of 2.17 (Handel et al., 2010). This study adds weight to this evidence, and benefited from a substantially larger study population than most previous individual case-control studies. In terms of exposure to infectious mononucleosis in MS and controls our results were similar to a smaller study using an earlier version of the CPRD database, which found that 2.2% of MS patients and 0.7% controls had been exposed (Marrie et al., 2000).

Previous infectious mononucleosis and increasing latitude are both risk factors for MS, and it had been hypothesised that vitamin D deficiency at higher latitudes may modulate the immune response to EBV, such that the risk of subsequently developing MS is increased. However, similar to Lossius et al. (2014), we did not find that infectious mononucleosis in winter, when serum vitamin D levels are at their lowest, was more strongly associated with MS than infectious mononucleosis during summer. Although the odds ratios were about one, the confidence intervals were wide. Therefore, although we have excluded a two-fold increase in the odds of MS with

winter exposure, there may be a smaller but still significant association. Larger studies would be needed to detect this. Lossius et al. (2014) found higher overall rates of prior infectious mononucleosis exposure than we did in both MS patients (14%) and controls (7.2%). This may be because they overestimated exposure because of inaccurate self-reporting by patients and we underestimated exposure due to missed reporting in the CPRD.

The strength of this study was the use of a large unselected national primary care database with medical confirmation (not self-reporting) of the diagnoses of MS and infectious mononucleosis. The main limitations were due to issues with the coded data. 87% of patients lacked a clear date for their first attack of MS, which meant that it is possible that infectious mononucleosis had occurred after the onset of their MS. However, this is unlikely given that the mean age of diagnosis of infectious mononucleosis was 21 years in MS patients, well before the usual age of onset of MS. Many patients with infectious mononucleosis also had to be excluded because they lacked an accurate date of diagnosis (three had no date recorded and 488 had 1st January recorded, which is likely to have been an automatically generated date when only the year of infectious mononucleosis infection, rather than the full date, had been entered into the CPRD database). Exclusion of these infectious mononucleosis cases resulted in reduced power in our study but we do not believe it introduced a bias as the excluded cases were roughly evenly distributed amongst MS patients and controls. It would have required the missing 128 MS patients to have a 2:1 ratio in winter:summer infectious mononucleosis exposure to generate a statistically significant odds ratio if the missing controls had a similar winter:summer ratio as the analysed controls. We also inferred vitamin D status from the season of exposure as

we did not have measurements of actual vitamin D status at the time of infectious mononucleosis exposure, which would be difficult to ascertain.

We found no effect of seasonality of infectious mononucleosis on the risk of developing MS. If vitamin D does interact with EBV infection to alter the risk of MS, it may be that the immunoregulatory activity of vitamin D plays a more important role during latent EBV infection, rather than during the initial infection.

In conclusion, this case-control study confirmed the findings of earlier studies that previous infectious mononucleosis is a risk factor for MS. However, there was no evidence of an association between the season of infectious mononucleosis infection and subsequent risk of MS. It would be useful to corroborate our findings in other large scale case-control or cohort studies with more detailed timing of infectious mononucleosis and the onset of MS.

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REFERENCES

Almohmeed, Y.H., Avenell, A., Aucott, L., Vickers, M.A., 2013. Systematic review and meta-analysis of the sero-epidemiological association between Epstein Barr virus and multiple sclerosis. *Plos One.* 8(4), e61110. doi: 10.1371/journal.pone.0061110.

Aranow, C., 2011. Vitamin D and the immune system. *J. Investig. Med.* 59, 881–886. doi: 10.231/JIM.0b013e31821b8755.

Ascherio, A., Munger, K.L., 2007a. Environmental risk factors for multiple sclerosis. Part II: non infectious factors. *Ann. Neurol.* 61, 504-513.

Ascherio, A., Munger, K.L., 2007b Environmental risk factors for multiple sclerosis. Part I: the role of infection. *Ann. Neurol.* 61, 288-299.

CPRD homepage. https://www.cprd.com/home/ (accessed 17.07.2016).

Handel, A.E., Williamson, A.J., Disanto G., Handunnetthi, L., Giovannoni G., Ramagopalan S.V., 2010. An updated meta-analysis of risk of multiple sclerosis following infectious mononucleosis. *Plos One.* 5(9), pii, e12496. doi: 10.1371/journal.pone.0012496.

Holmøy, T., 2008. Vitamin D status modulates the immune response to Epstein Barr virus: Synergistic effect of risk factors in multiple sclerosis*. Med. Hypotheses*. 70, 66-69.

Hypponen, E., Power, C., 2007. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. *Am. J. Clin. Nutr.* 85, 860-868.

James, E., Dobson, R., Kuhle, J., Baker D., Giovannoni G., Ramagopalan S.V., 2013. The effect of vitamin D-related interventions on multiple sclerosis relapses: a meta-analysis. *Mult. Scler.* 19, 1571-1579. doi: 10.1177/1352458513489756.

Levin, L.I., Munger, K.L., et al., 2005. Temporal relationship between elevation of epstein-barr virus antibody titers and initial onset of neurological symptoms in multiple sclerosis. *JAMA.* 293, 2496-2500.

Lossius, A., Riise, T., et al., 2014. Season of infectious mononucleosis and risk of multiple sclerosis at different latitudes; the EnvIMS Study. *Mult Scler.* 20, 669-674. doi: 10.1177/1352458513505693.

Marrie, R.A., Wolfson, C., et al., 2000. Multiple sclerosis and antecedent infections: a case-control study. *Neurology.* 54, 2307-2310.

Marshall, N.A., Vickers, M.A., Barker, R.N., 2003. Regulatory T cells secreting IL-10 dominate the immune response to EBV latent membrane protein 1. *J. Immunol.* 170, 6183-6189.

Marshall, N.A., Culligan, D.J., Johnston, P.W., Millar C., Barker R.N., Vickers M.A., 2007. CD4+ T-cell responses to Epstein-Barr virus (EBV) latent membrane protein 1 in infectious mononucleosis and EBV associated non-Hodgkin lymphoma: Th1 in active disease but Tr1 in remission. *Br. J. Haematol.* 139, 81-89.

Munger, K.L., Levin, L.I., Hollis, B.W., Howard N.S., Ascherio A., 2006. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA.* 296, 2832–2838.

Thacker, E.L., Mirzaei, F., Ascherio, A., 2006. Infectious mononucleosis and risk for multiple sclerosis: a meta analysis. *Ann. Neurol.* 59, 499-503.

UK Climate Period: 1981-2010 - Sunshine (hours). http://www.metoffice.gov.uk/public/weather/climate/gcpvn15h9%20-%20?tab=climateGraphs. (accessed 17.07.16).

Figure legend

Figure 1: Study flow diagram

Figure footnotes:

CPRD = Clinical Practice Research Database;

MS = multiple sclerosis;

IM = infectious mononucleosis.