Evaluation of Prolonged Fatigue Post–West Nile Virus Infection and Association of Fatigue with Elevated Antiviral and Proinflammatory Cytokines

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Abstract

This study aimed to characterize fatigue postinfection among study participants with a history of West Nile virus (WNV) infection and determine whether antiviral and pro-inflammatory cytokines were significantly elevated in those reporting prolonged fatigue. We found that 31% (44/140) of study participants experienced prolonged (more than 6 months) fatigue postinfection, with an average length of fatigue duration of 5 years. Females, those younger than 50 years of age, and those with symptomatic clinical WNV disease were significantly more likely to report fatigue. Pro-inflammatory and antiviral cytokines (granulocyte macrophage colony stimulating factor, interferon- γ , interferon- γ inducing protein 10, interleukin 2, interleukin 6, and interleukin 12p70) were significantly elevated in those reporting fatigue postinfection compared to those not reporting fatigue. Clinicians should consider history of WNV infection as a possible factor when evaluating causes of prolonged fatigue following a febrile viral illness in their patients.

Introduction

WEST NILE VIRUS (WNV) is a disease transmitted by mosquitoes that has infected approximately three million adults across the United States (3). While most infected individuals do not develop any signs or symptoms of disease, roughly 20% will develop febrile illness, and less than 1% will develop neuroinvasive disease, characterized as encephalitis, meningitis, and/or acute flaccid paralysis (17). Symptomatic WNV patients are likely to suffer from extended morbidity and mortality several years postinfection, particularly neurologic impairment (2,14,15). Common outcomes include short-term memory loss, concentration impairment, muscular weakness, and fatigue (2). Debilitating fatigue can be a substantial outcome of the disease, resulting in both personal and economic tolls (21,34).

In Houston, we have been following a cohort of study participants with a history of WNV infection since the initial outbreak in 2002. Subjectively, participants continued to report prolonged fatigue following infection, with 20% of study participants reporting fatigue up to 8 years later (20). Additionally, we recently reported the persistence of anti-WNV IgM antibodies in 23% of study participants 8 years following infection (19). We hypothesized that persistent ac-

tivation of the pro-inflammatory innate immune response could be responsible for fatigue in our study population. To test this hypothesis, we evaluated fatigue and compared cytokine profiles to determine if pro-inflammatory cytokines were elevated in those identified with fatigue. With recent evidence of persistent WNV infection, it is imperative that we understand all possible long-term effects of this disease (18). This paper presents the findings of our objective evaluation of fatigue, symptomology, and associated cytokine profiles among study participants with a history of WNV infection.

Methods

A cohort of WNV participants was established in 2002 in Houston, Texas, and followed prospectively from the time of acute infection to time of evaluation (1–10 years post-infection). Study participants were originally identified through local health department surveillance or routine screening of the blood supply at local blood donation centers during 2002–2011. Study protocol and procedures were approved by the University of Texas Health Science Center and the Baylor College of Medicine institutional review boards. To date, 220 participants have consented and have

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enrolled into the cohort. From the original cohort, 61 were excluded from this study based on the following criteria: 24 deceased, 11 determined a false positive case for WNV, 24 lost to follow-up, and/or 6 refusing to continue long-term participation. Of the 159 study participants eligible for this study, 140 took part. The evaluation took place from April 2011 to July 2012 and included a questionnaire and blood donation.

Questionnaire

For the purposes of this study, fatigue postinfection was defined as fatigue that impaired one's daily activities for 6 months or more following, but not prior to, infection with WNV. Participants self-reported signs and symptoms, onset, and duration of fatigue. Since fatigue postinfection had not been previously examined among this population, we used the Centers for Disease Control and Prevention's (CDC) chronic fatigue syndrome case definition symptom criteria and list of alternative symptoms related to fatigue as a guideline for characterizing additional fatigue-related symptoms (10). Participants also completed the Modified Fatigue Impact Scale (MFIS), a validated questionnaire that measures the impact of fatigue on physical, cognitive, and psychosocial functioning (32). Lastly, participants self-reported conditions that might also influence having fatigue, including chronic medical conditions and height and weight for body mass index calculations.

Descriptive statistics were used to describe the prevalence of fatigue postinfection, symptoms, and comorbidities among the cohort. Univariate logistic regression analysis was performed to assess potential associations between symptoms, demographics, and comorbidities with having or not having fatigue postinfection. Multivariate logistic regression analysis was used to identify independent associated factors and to test for potential confounding. All associated factors on univariate analysis with $p \leq 0.25$ were included in multivariate analysis. A backwards step elimination of the highest nonsignificant value method was used. Only those factors with $p \leq 0.10$ were considered significant in the multivariate analysis. Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks was performed to validate fatigue status with the MFIS scores.

Cytokine analysis

A blood sample was collected on 77 randomly selected participants and used for cytokine analysis. A cytokine panel composed of 20 antiviral and/or pro-inflammatory cytokines, chemokines, and growth factors was performed using a Luminex® IS 100 platform (Austin, TX) with Miliplex Analyst software. Histogram analysis found that all variables were skewed; therefore median concentrations (pg/mL) and standard deviations were reported. Wilcoxon rank-sum test was performed to look at statistical differences in median concentrations between the fatigue and nonfatigue groups. For the rank-sum and chi-square analyses, those with $p < 0.05$ were considered significant.

Results

Among a cohort of participants with a history of WNV infection, 31% (44/140) reported experiencing fatigue post-

infection that inhibited their daily activities. Of those reporting fatigue postinfection, all reported fatigue at the time of evaluation, and 70% recalled experiencing fatigue immediately following initial infection. The average length of fatigue duration was 5 years with a range of 6 months to 10 years. Of those with fatigue postinfection, 28 (64%) met the CDC's case definition of chronic fatigue syndrome (10).

As seen in Figure 1, Kruskal–Wallis one-way ANOVA on ranks found the median scores on the MFIS were significantly higher ($\chi^2 = 41.3$; $p < 0.0001$) among those with fatigue postinfection (median = 44) compared to those without reported fatigue (median = 9). The prevalence of the 19 symptoms significantly associated with fatigue postinfection is listed in Table 1. Symptoms of feeling tired or unwell after exertion, impaired memory or concentration, unrefreshing sleep, morning stiffness, and multiple arthralgias were the most commonly reported.

Logistic regression was performed to analyze differences in demographics and comorbidities between those with and without fatigue postinfection (Table 2). On univariate analysis, we found the following variables significant: male gender, asymptomatic WNV infection, and non-neuroinvasive West Nile fever. In addition, being aged ≥ 50 years ($p = 0.06$) was found to be appropriate for inclusion in the multivariate analysis. After performing multivariate analysis and model building strategies, we found the following variables to be significantly associated with fatigue postinfection: female gender ($p = 0.049$), younger than 50 years of age ($p = 0.03$), and symptomatic WNV infection ($p = 0.003$).

Serum samples were available for cytokine analysis on 61% (25/41) of those reporting fatigue and on 53% (52/99) of those not reporting fatigue postinfection. All variables were found to be skewed. Therefore, we performed a Wilcoxon rank-sum analysis of cytokines by fatigue status as shown in Table 3. Interleukin (IL)-12p70, granulocyte macrophage-colony stimulating factor (GM-CSF), IL-2, and interferon- γ (IFN- γ) were significantly elevated ($p < 0.01$) in those reporting fatigue compared to those not reporting fatigue postinfection. IL-6 and interferon γ -inducing protein 10 (IP-10) were also significantly elevated ($p < 0.05$) in those reporting fatigue compared to those not reporting

FIG. 1. Modified Fatigue Impact Scale scores by prolonged fatigue status reported post–West Nile virus infection.

TABLE 1. FATIGUE POSTINFECTION SYMPTOM PREVALENCE

	<i>Postinfection fatigue, n = 44 (%)</i>
Impaired memory or concentration	36 (82)
Tired or unwell after exertion	33 (75)
Unrefreshing sleep	33 (75)
Morning stiffness	24 (55)
Multiple arthralgias	22 (50)
Bloating	21 (48)
Persistent muscle pain	20 (45)
Night sweats	20 (45)
Headaches	20 (45)
Skin sensations	17 (39)
Shortness of breath	15 (34)
Dizziness	15 (34)
Psychological problems (anxiety and/or depression)	14 (32)
Abdominal pain	10 (23)
Nausea	10 (23)
Irregular heartbeat	9 (20)
Tender lymph nodes	9 (20)
Jaw pain	8 (18)
Diarrhea	7 (16)

Only those symptoms found significantly ($p < 0.05$) associated with fatigue postinfection are listed.

fatigue postinfection. Lastly, IL-10, IL-8, IL-15, IFN- α , and macrophage inflammatory protein (MIP)-1 β neared significance ($p < 0.10$) and were all elevated in the fatigue group. We did not find any statistical associations by rank-sum related to downregulation of cytokines.

When examining the six cytokines (GM-CSF, IFN- γ , IP-10, IL-2, IL-6, and IL-12p70) that were found to be significant on the Wilcoxon ranksum analysis, we examined the actual concentrations (pg/mL) and used the 95% upper confidence limit (UCL) as a cutoff value for determining which participants had a higher than expected level of cy-

tokine concentration (Table 4). With the exception of IP-10, we found that those with fatigue were significantly more likely to have a higher proportion of participants with cytokine levels at or above the 95% UCL when compared to those without fatigue.

Discussion

We found that 31% of the cohort reported fatigue postinfection for at least 6 months duration, which is considerably higher than the U.S. prevalence of $< 1\%$ for chronic fatigue (6). Understanding the mechanisms of fatigue are particularly important considering that chronic fatigue can result in an annual household income loss of \$20,000 (34). The average length of fatigue duration was 5 years in our population, which if unresolved could result in substantial long-term personal and economic tolls. Females, those younger than 50 years of age, and those with symptomatic clinical WNV disease were the most likely to suffer from fatigue postinfection in our population. Other studies also found females and severity of disease during the acute phase of infection to be associated with prolonged morbidity from WNV (2,34). However, this is the first study to report younger age being associated with prolonged fatigue. Nineteen symptoms were significantly associated with WNV fatigue postinfection, and presence of these symptoms should be used as a guideline for evaluating this condition.

We found that in our population of persons with a history of WNV, those reporting fatigue postinfection had a significant elevation of pro-inflammatory and antiviral cytokines compared to those without fatigue postinfection. Cell culture studies have demonstrated that for acute WNV infection, tumor necrosis factor (TNF)- α and IL-1 β are upregulated during viral replication, IL-6 and TNF- β are upregulated during viral clearance from macrophages, and IP-10 is upregulated in macrophages throughout the course of infection (29). We found TNF- α and IL-1 β to be neither significantly elevated nor decreased in our fatigue population. However, we did find IL-6, TNF- β , and IP-10

TABLE 2. DEMOGRAPHICS AND COMORBIDITIES OF PARTICIPANTS BY WNV FATIGUE POSTINFECTION STATUS

	<i>Reporting fatigue postinfection, n = 44 (%)</i>	<i>Not reporting fatigue postinfection, n = 96 (%)</i>	<i>Univariate odds ratio (95% CI)</i>	<i>p-Value</i>	<i>Multivariate odds ratio (95% CI)</i>	<i>p-Value</i>
<i>Demographics</i>						
Male gender	19 (43)	61 (64)	0.44 (0.21–0.90)	0.03	0.46 (0.21–0.99)	0.049
Age 50 years and older	25 (57)	70 (73)	0.49 (0.23–1.03)	0.06	0.40 (0.17–0.92)	0.032
Non-Hispanic white race	38 (86)	81 (84)	REFERENCE			
Black race	2 (5)	9 (9)	0.47 (0.10–2.30)	0.35		
Hispanic ethnicity	2 (5)	4 (4)	1.07 (0.19–6.08)	0.94		
Other race	2 (5)	2 (2)	2.13 (0.29–15.71)	0.46		
<i>Comorbidities</i>						
History of Neuroinvasive WNV	22 (50)	44 (46)	1.18 (0.58–2.41)	0.65		
History of WNF (non-neuroinvasive)	20 (45)	26 (27)	2.24 (1.07–4.73)	0.03		
History of Asymptomatic WNV	2 (5)	26 (26)	0.13 (0.03–0.57)	0.007	0.09 (0.02–0.44)	0.003
Hypertension	17 (39)	31 (32)	1.32 (0.63–2.77)	0.46		
Diabetes mellitus	6 (14)	11 (11)	1.22 (0.42–3.54)	0.72		
Overweight or obese (BMI ≥ 25)	27 (61)	57 (59)	0.95 (0.45–1.99)	0.89		

WNV, West Nile virus; WNF, West Nile fever; BMI, body mass index.

TABLE 3. WILCOXON RANK-SUM ANALYSIS OF ANTIVIRAL AND PRO-INFLAMMATORY CYTOKINES BY FATIGUE STATUS

<i>Cytokines</i>	<i>p-Value</i>	<i>With postfatigue median $\pm$ standard deviation</i>	<i>Without postfatigue median $\pm$ standard deviation</i>
Granulocyte macrophage colony stimulating factor	0.003	5.57 $\pm$ 87.36	3.25 $\pm$ 6.80
Interferon α	0.080	21.92 $\pm$ 66.97	14.46 $\pm$ 27.35
Interferon γ	0.009	8.12 $\pm$ 169.71	5.09 $\pm$ 28.14
Interferon γ -Induced Protein 10	0.043	455.00 $\pm$ 316.10	342.00 $\pm$ 163.24
Interleukin 1 α	0.318	14.82 $\pm$ 192.23	12.15 $\pm$ 59.64
Interleukin 1 β	0.328	2.73 $\pm$ 48.57	1.95 $\pm$ 10.97
Interleukin 2	0.007	2.05 $\pm$ 65.13	1.59 $\pm$ 1.80
Interleukin 3	0.137	1.56 $\pm$ 1.50	1.56 $\pm$ 0.381
Interleukin 4	0.193	35.87 $\pm$ 55.74	23.52 $\pm$ 31.06
Interleukin 5	0.345	1.26 $\pm$ 2.51	1.05 $\pm$ 1.75
Interleukin 6	0.025	5.77 $\pm$ 41.18	2.86 $\pm$ 4.99
Interleukin 7	0.513	4.60 $\pm$ 6.69	4.80 $\pm$ 23.39
Interleukin 8	0.067	26.56 $\pm$ 191.19	16.77 $\pm$ 94.94
Interleukin 10	0.064	7.97 $\pm$ 9.55	5.70 $\pm$ 7.20
Interleukin 12p70	0.002	7.36 $\pm$ 193.36	3.12 $\pm$ 19.40
Macrophage inflammatory protein 1 α	0.337	5.32 $\pm$ 36.00	5.14 $\pm$ 12.48
Macrophage inflammatory protein 1 β	0.097	63.58 $\pm$ 85.85	53.35 $\pm$ 40.21
Monocyte chemotactic protein 1	0.248	519.00 $\pm$ 218.48	558.50 $\pm$ 259.77
Tumor necrosis factor α	0.804	12.54 $\pm$ 25.69	11.78 $\pm$ 5.53
Tumor necrosis factor β	0.330	4.51 $\pm$ 22.57	3.36 $\pm$ 10.37

to be elevated. This suggests that fatigue symptoms might be a result of one's body trying to clear residual stores of virus or some viral component via monocytic immune cell lineages, although the virus might no longer be actively replicating.

In addition to WNV-related cytokines, we also found upregulation of other pro-inflammatory and antiviral cytokines. GM-CSF, IFN- γ , and IL-8 contribute to an inflammatory response by recruiting macrophages and other innate immune cells to the site of infection (7,12). Their upregulation in our fatigue group suggests an ongoing immune response, and some synergistic action toward controlling the underlying cause of inflammation. Whether WNV or a viral antigen is the root cause of this inflammation is unknown. However, the increased levels of antiviral cytokines IFN- α , and IFN- γ do suggest that a viral component is present, raising the possibility of an induced postviral autoinflammatory state.

Cytokine dysregulation, particularly increased presence of pro-inflammatory cytokines, is believed to be a main contributing factor to chronic fatigue syndrome pathogenesis. While our fatigue population has similar cytokine profiles to those with chronic fatigue syndrome, we did find some distinctions. TNF- α and TNF- β has been indicated as a marker of disease for those with chronic fatigue syndrome (16,23). We did not find either to be significantly different between those with and without fatigue in our population, which could be due to differing immune modulators between our fatigue postinfection patients and those with chronic fatigue syndrome. It also speaks to the potentially unique nature of the syndrome described here in association with WNV infection, or perhaps a feature of temporal sampling in our cohort relative to other immunological studies of fatigue.

Our analysis found elevated IL-12p70, IP-10, and IL-6 positively associated with fatigue. IL-3 and IL-6 can work in tandem to help stimulate production of myeloid dendritic

cells, which then mature in the presence of GM-CSF (8). We believe the significant elevation of these three cytokines in our fatigue group potentially represents that this pathway is active and causing a stimulation of innate immune cells. This theory is further supported by the significant findings of pro-inflammatory cells IL-12p70 and IP-10 being elevated. IL-12p70 is the bioactive form of IL-12 produced by dendritic cells and macrophages, which then plays a primary role in development of cell-mediated immunity by activating IFN- γ (11). We found significantly increased levels of IFN- γ and IP-10, further suggesting a successful inflammatory response is occurring in our fatigue population.

In our population with past WNV infection, roughly one-third reported having debilitating prolonged fatigue that statistically correlated with elevated pro-inflammatory and antiviral cytokines. Extended viral infection could be a cause of this fatigue. Studies have found that an underlying infection or a viral reactivation triggering a chronic immune response can result in symptoms of chronic fatigue (1,5,13,16,27,34). This has particularly been demonstrated by neuropathogens (13). WNV causes a neuroinvasive disease in a small percentage of those infected (17) and can result in long-term mental and physical impairments (2,14,21). Extended IgM antibody titers have been documented several years postinfection in humans (22,26,28), and persistent neurologic and renal infection has been documented in animal models (25,30,31). Previous findings in this cohort have found persistence of IgM antibodies in 42%, 34%, and 23% of WNV-positive study participants approximately 1, 6, and 8 years postinfection, respectively (19). However, we did not find any statistical correlations between IgM antibodies and fatigue in this study. Based on the mounting evidence of persistent viral causes of chronic fatigue with neuropathogens similar to WNV, we cannot rule out the possibility a prolonged WNV infection resulting in a chronic inflammatory response is leading to fatigue.

TABLE 4. ANALYSIS ON THE SIX CYTOKINES FOUND TO BE SIGNIFICANT BETWEEN WNV-POSITIVE PATIENTS WITH AND WITHOUT FATIGUE

Cytokines	Range	Median	95% lower confidence limit (LCL)	95% upper confidence limit (UCL)	No. of participants without post-WNV fatigue with values $\geq 95\%$ UCL, n = 57 (%)	No. of participants with post-WNV fatigue with values $\geq 95\%$ UCL, n = 25 (%)	Chi-square	p-Value
Granulocyte macrophage colony stimulating factor	0.8–440.0	3.71	3.3	5.0	16 (28)	14 (56)	4.52	0.03
Interferon γ	2.4–589.0	6.69	5.09	7.64	17 (30)	15 (60)	5.18	0.02
Interferon γ -inducing protein 10	148.0–1615.0	356.0	326.0	414.0	17 (30)	13 (52)	2.65	0.10
Interleukin 2	1.59–311.0	1.59	1.59	1.75	16 (28)	14 (56)	4.52	0.03
Interleukin 6	1.19–179.0	3.19	2.07	5.57	16 (28)	14 (56)	4.52	0.03
Interleukin 12p70	2.84–932.0	7.36	3.6	25.53	4 (7)	8 (32)	7.58	0.006

We found a substantially higher prevalence of fatigue among our study population (31%) compared to what has been previously published with regard to the worldwide prevalence of chronic fatigue syndrome as 0.4–1% (6,34). A recent statistic published by the Centers for Disease Control and Prevention's National Health Interview Survey (NHIS) reported that 15% of adult women and 10% of adult males reported feeling very tired or exhausted over the 3 months preceding the interview (4). In our study, we found more than a twofold increase in prevalence of reported fatigue when compared to the NHIS, with 42% of females and 24% of males reporting debilitating fatigue for a period of 6 months or more. It appears that WNV female survivors are at an increased risk for suffering from prolonged fatigue similar to other studies (9,21).

Of those who had fatigue postinfection, 95% (42/44) had symptomatic WNV infection, with roughly half being febrile cases. In fact, non-neuroinvasive febrile WNV disease was significantly ($p=0.03$) associated with having fatigue postinfection on univariate analysis, while neuroinvasive WNV disease was not found to be significant. Non-neuroinvasive febrile WNV cases largely go undiagnosed in the United States, theoretically due to lack of testing and underreporting by physicians (3). The strong correlation of extended fatigue in our population suggests that prior WNV infection could be an influential factor in those with diagnosed chronic fatigue syndrome. If the underlying cause is not known, physicians should consider testing their patients with diagnosed chronic fatigue syndrome for previous WNV infection, particularly if the patient reports a history of febrile viral-like illness prior to the onset of fatigue.

A recognized limitation of the study was that fatigue diagnosis was based on self-reported symptoms. However, the MFIS has been shown to be a reliable indicator of fatigue in other studies, and we were able to validate fatigue in our cohort population using this scale (32,33). Studies are underway to verify these results by comparing to an age-, gender-, and race/ethnicity-matched WNV-negative control population. Despite our limitations, we found statistical differences between those with and without symptoms of fatigue reported postinfection by validated MFIS scales and cytokine analysis. Further studies are needed to understand the immunologic and biomechanical factors underlying the debilitating fatigue in our study population.

Conclusion

This study aimed to characterize the symptomology and cytokine profile of those reporting fatigue postinfection among participants with a history of WNV infection. We found that 31% reported having fatigue postinfection with an average duration of 5 years. Being female, having symptomatic WNV disease, and being younger than 50 years of age were all found to be independently associated with fatigue postinfection. Symptoms of feeling tired or unwell after exertion, impaired memory or concentration, unrefreshing sleep, morning stiffness, and multiple arthralgias were the most commonly reported symptoms that were statistically associated with having fatigue postinfection. Pro-inflammatory and antiviral cytokines (GM-CSF, INF- γ , IP-10, IL-2, IL-6, and IL-12p70) were significantly elevated in those reporting fatigue postinfection compared to those not reporting fatigue. We cannot

rule out the influence of WNV on elevated pro-inflammatory cytokines resulting in fatigue postinfection. Clinicians should consider history of symptomatic WNV infection as a possible factor when evaluating causes of fatigue in their patients.

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