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Pregnant women infected with pandemic influenza A(H1N1)pdm09 virus showed differential immune response correlated with disease severity

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ABSTRACT

Background: During pregnancy, immunological and hormonal alterations place women at increased risk for influenza-related severe illnesses including hospitalization and death. Although A(H1N1) pdm09 infection resulted in increased disease severity in pregnant women, the precise mechanisms responsible for this risk have yet to be established.
Objectives: The present study was aimed to investigate the role of host chemokines and cytokine profiles in A(H1N1) pdm09 infection regarding disease severity in pregnant women.
Study design: This retrospective survey examined 41 pregnant women with confirmed A(H1N1) pdm09 infection. Of them, 12 died (D), 29 survived (S), and 17 remained uninfected and served as controls (C). Antiviral response was evaluated for IFN-β expression and gene expression profiles of cytokines (TNF-α, IL-6, IL-12, TGF-β) and chemokines (IL-8, RANTES, MCP-1, IP-10), and the viral Matrix (M1) gene was quantified and normalized using the housekeeping gene product β-actin mRNA.
Results: Higher IL-8 and TNF-α mRNA expression were found in D and S compared with C, while IL-6 showed higher expression in D. Interestingly, these results were associated with a decrease in the anti-inflammatory response of TGF-β mRNA and IFN-β. These alterations would lead to an imbalance in the immune response of those patients.
Conclusions: Pregnancy-related reductions in IFN-β and TGF-β expression levels and elevated levels of pro-inflammatory cytokines could explain the increased severity of infection and death of pregnant women. These findings may help improve the understanding of the high susceptibility and disease severity to influenza virus infection during pregnancy.

1. Background

Increased morbidity and mortality rates over the course of pregnancy have been documented all through pandemic influenza and seasonal influenza where virus infection rates are particularly high [1]. Pregnancy has been associated with decreased inflammatory responses and stable/increased anti-inflammatory responses to immune challenges in women as well as in animal models [2–7]. Abnormalities in immune adaptation to pregnancy may affect pregnancy outcomes such as risk of preeclampsia, poor fetal growth, and preterm birth [8–11]. Many clinical studies have been performed to empirically define the increased severity of influenza infections during pregnancy. Epidemiological evidence and animal studies have shown that influenza infections are more severe in the second and third trimesters of pregnancy, resulting in greater morbidity and mortality, although the reason for this is still unclear [1,12,13].

Immunological alterations during pregnancy may help explain the increased severity of and susceptibility to infectious diseases.

Abbreviations: A(H1N1)pdm09, pandemic influenza H1N1, 2009; IL, interleukin; IFN-β, interferon beta; MCP-1, monocyte chemoattractant protein 1; TNF-α, tumor necrosis factor-alpha; TGF-β, transforming growth factor-β.

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As pregnancy progresses, hormone levels change dramatically and are considerably higher than those in nonpregnant females [14]. The interplay between sex hormones and the immune system is complex and multifactorial, affecting thus many organ systems.

The increased morbidity due to influenza infection is associated with higher levels of circulating estrogen. Estrogens have long been known to possess potent immunomodulatory effects in various models of disease [15–17].

The innate immune system, the first line of defense against invading viruses, involves two types of cytokine responses: a pro-inflammatory response and an antiviral response.

Inflammatory cytokines and chemokines play a key role in the pathogenesis of virus infections [18]. Influenza viruses primarily infect the epithelial cells of the upper respiratory tract, evoking release of an array of host inflammatory and antiviral cytokines and chemokines and the recruitment of antiviral immune cells to the infection site.

The increased mortality rate detected in pregnant female mice infected with influenza A(H1N1)pdm09 virus is associated with increased infiltration of neutrophils and macrophages in the lungs of these animals. In addition, pregnant mice showed higher levels of chemokines and pro-inflammatory cytokines, lower respiratory epithelial regeneration and poorer fetal development than non-pregnant mice [19,20].

The increased mortality rate observed in pregnant female mice correlates with greater induction of pro-inflammatory cytokines and chemokines, including TNF-α, MPC-1 in the lungs following infection [20,21].

On the other hand, respiratory epithelial cells have been shown to produce antiviral cytokines such as interferon (IFN-β) [22]. *In vitro* data showed that pregnant women have an attenuated innate interferon response in peripheral blood mononuclear cells (PBMCs) stimulated with A(H1N1)pdm09 compared with PBMCs from nonpregnant women [23].

Despite improvements in healthcare, the bad prognosis of influenza virus infection during pregnancy remains a health concern, as most recently demonstrated by the 2009 influenza virus A subtype H1N1 (H1N1/09) outbreak.

In this study, we hypothesized that the alterations in the pro-inflammatory response and antiviral response may contribute to the severity of the infection in pregnant women infected with A(H1N1)pdm09.

2. Objectives

In an attempt to elucidate the innate immune response to A(H1N1) pdm09 infection and to gain further insight into cytokine-mediated pathogenesis, we retrospectively evaluated the expression levels of a panel of cytokines, chemokines, and viral replication in different groups of pregnant women according to the severity of the infection.

3. Study design

3.1. Subjects and samples

Samples were nasopharyngeal swabs collected from July to September 2009 and sent to the National Influenza Reference Laboratory which houses the WHO National Influenza Center (NIC) for A(H1N1)pdm09 diagnosis.

This study included 41 pregnant women with confirmed A(H1N1)pdm09 infection during the 2009 pandemic. Of them, 29 survived (*S*), and 12 died as a result of infection (*D*). Cardio-respiratory failure was the leading cause of death. Samples of 17 pregnant women non infected with influenza virus A or B were included as control (*C*); this group was enrolled as non-influenza acute respiratory infections (ARI). Samples were obtained in the second and third trimester of pregnancy, because susceptibility and severity of influenza virus infection increases with gestational age [13,24].

Samples were collected with an average of 4.5 days after onset of symptoms for all groups.

This study was approved by the Independent Ethics Committee of the School of Medicine, University of Buenos Aires, Argentina, informed of internationally endorsed standards for the application of the Helsinki Declaration.

Table 1
Primers, probes sequences, and quantitative PCR conditions.

	Sequence		Annealing temperature	Cycle number	Size (bp)
β-Actin	Forward	5′-ATGGGTCAGAAGGATTCCTATGTG-3′	60	40	435
	Reverse	5′-CTTCATGAGGTAGTCAGTCAGGTC-3′			
RANTES	Forward	5′-GTCGTCTTTGTCACCCGAAAG-3′	60	40	65
	Reverse	5′-TCCCGAACCCATTTCTTCTCT-3′			
MCP-1	Forward	5′-CAAACTGAAGCTCGCACTCTCGCC-3′	60	40	354
	Reverse	5′-ATTCTTGGGTTGTGGAGTGAGTGTTCA-3′			
IL-8	Forward	5′-ACTGAGAGTGATTGAGAGTGGAC-3′	60	40	112
	Reverse	5′-AACCCTCTGCACCCAGTTTTC-3′			
IL-6	Forward	5′-TCCACAAGCGCCTTCGGTCCAG-3′	60	40	191
	Reverse	5′-CTCAGGGCTGAGATGCCGTCG -3′			
TNF-α	Forward	5′-CCCAGGCAGTCAGATCATCTTC -3′	60	40	85
	Reverse	5′- AGCTGCCCCTCAGCTTGA -3′			
IL-12	Forward	5′- TGTCACCGAGAAGCTGATGT-3′	60	40	278
	Reverse	5′-GAGGTTTCTGGCCAAACTGA-3′			
IL-10	Forward	5′- TTACCTGGAGGAGGTGATGC -3′	60	40	285
	Reverse	5′- GCCACCCTGATGTCTCAGTT -3′			
TGF-β	Forward	5′-GGACACCAACTATTGCTTCAG-3′	60	40	159
	Reverse	5′-TCCAGGCTCCAAATGTAGG-3′			
IFN-β	Forward	5′-CTTACAGGTTACCTCCGAAACTGAA-3′	60	40	80
	Reverse	5′-GGTTGAAGAATGCTTGAAGCAA-3′			
IP-10	Forward	5′-ATTATTCCTGCAAGCCAATTTTG-3′	60	40	65
	Reverse	5-TCACCCTTCTTTTTCATTGTAGCA-3			
M	Forward	5′- ATTATTCCTGCAAGCCAATTTTG -3′			
	Reverse	5′-AGGGCATTYTGGACAAAKCGTCTA-3′	50	45	244
	PROBE	5′-TGCAGTCCTCGCTCACTGGGCACG-3′			

M, Matrix gen; bp, base pairs.