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Prenatal Stress, Depression, and Herpes Viral Titers

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by

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DEDICATION

To my husband Shu-Ping for supporting and encouraging me to successfully accomplish my academic goal. I could not have finished this without you. To my two sons Andy and Michael for their support and understanding that their mother spends more time studying than being with them.

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LIST OF ACRONYMS

AA	African American
ANOVA	Analysis of variance
AIDS	Acquired immunodeficiency syndrome
β	Standardized regression coefficients
BCG	Bacilli Calmette-Guerin
BH4	5, 6, 7, 8-tetrahydrobiopterin
BH2	Quinonoid 7, 8-dihydrobiopterin
BMI	Body mass index
CI	Confidence interval
CMV	Cytomegalovirus
CO	Cut-off control
CRP	C-reactive protein
CTLA-4	Cytotoxic T lymphocyte antigen-4
DC	Dendritic cell
df	Degree of freedom
DSM	Diagnostic and Statistical Manual of Mental Disorder
EA	Early antigen
EBNA	Epstein-Barr virus nuclear antigen
EBV	Epstein-Barr virus

ELISA	Enzyme-linked immunosorbent assay
HHV-6	Human herpes virus -6
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigens
HPA	Hypothalamic-pituitary-adrenal
HPLC	High-performance liquid chromatography
HSV	Herpes simplex virus
HSV-1	Herpes simplex virus type 1
HSV-2	Herpes simplex virus type 2
5-HT	5-hydroxytryptamine
GTP-CH1	Guanosine-triphosphate-cyclohydrolase-1
IFN-γ	Interferon-γ
IDO	Indoleamine-2, 3-dioxygenase
IE gene	Immediate early gene
IgG	Immunoglobulin G
IL	Interleukin
IRB	Institutional review board
KYN /TRP	Kynurenine /tryptophan
LAT	Latency-associated transcript gene
Log 10	Logarithm 10
LMP	Latent membrane protein
M	Mean

MIEP	Major immediate early promoter
mL	Milliliter
MØ	Macrophage
η^2	Eta squared
NF-κB	Nuclear factor- kappa B
NK cell	Natural killer cell
NMDA	N-methyl-D-aspartate
nmol/L	Nano moles/liter
NO	Nitric oxide
OD	Optical density
p	Probability value or Significant level
PAH	Phenylalanine 4-hydroxylase
Phe /Tyr	Phenylalanine /tyrosine
PI	Principle investigator
POMS	Profile of Mood States
POMS-D	Profile of Mood States-Depression
POMS-F	Profile of Mood States-Fatigue
PPD	Postpartum depression
PSS	Perceived Stress Scale
r	Correlation coefficient
R^2	R squared (Coefficient of determination)
ROS	Reactive oxygen species

rpm	Revolution per minute
SAM	Sympathoadrenomedullary
SD	Standard deviation
SPSS	Statistical Package for Social Sciences
t	t-test value
TDO	Tryptophan-2, 3-dioxygenase
Th1 cell	T helper -1 cell
Th2 cell	T helper -2 cell
TNF-α	Tumor necrosis factor-α
TPH	Tryptophan hydroxylase
TPO	Thyroid peroxidase antibody
µL	Microliter
µmol/L	Micromoles /liter
VCA	Viral capsid antigen

ABSTRACT

Recent studies suggest that some cases of prenatal depression may be associated with reactivation of latent infections of the herpesvirus family. The possible relationships among stress, prenatal depression, and herpes viral reactivation in pregnancy are understudied and the molecular pathways such as the neuroimmune biogenic amine pathway are unidentified. Chronic stress shifts the T helper-1 cell (Th1) cytokine profile to a Th2 profile, which favors virus induced pathogenesis and survival. Pregnancy is also associated with a similar Th2 dominance. In non-pregnant individuals, exposure to psychological or physical stress may be associated with latent herpes viral reactivation and could result in behavioral deficits and depression. Normally, type-1 cytokines such as Interferon-γ (IFN-γ) and inflammatory cytokines such as tumor necrosis factor-α (TNF-α) induce indoleamine-2, 3-dioxygenase (IDO) activation which inhibits herpes virus replication and reactivation, decreases tryptophan production, and alters phenylalanine /tyrosine metabolism. Thus it is possible that prenatal depression may occur from tryptophan stealing through the IDO pathway which results in decreased serotonin as well as increased risk for latent herpes viral reactivation.

The purpose of this study is to analyze the relationships among stress, herpes viral titers, depression, and metabolites of IDO activation, which involves tryptophan and guanosine-triphosphate-cyclohydrolase-1(GTP-CH1) pathways. This study builds on *Influence of Lactation on Postpartum Stress and Immunity* (Grant number: R01-NR05000) which investigated perinatal immune, endocrine, and inflammatory changes in pregnancy and the postpartum. A secondary

data analysis was conducted on baseline data from women collected at 16 to 25 gestational weeks. This data set included some herpes viral titers, and additional ones were measured in stored plasma samples. The aim of this study is to examine relationships among stress, herpes viral reactivation, depression, and the IDO activation pathway. The results of this study provide information about the possible role of further relationships of prenatal stress, latent herpes viral reactivation, and depression mechanisms. The results will be important in health promotion and disease prevention during pregnancy.

CHAPTER ONE

INTRODUCTION

Pregnancy is a vulnerable time for women. Physiological changes in pregnancy include a general suppression of type 1 immunity and a favoring of type 2 immunity along with a generalized but highly regulated inflammatory state (Mor & Cardenas, 2010; Rusterholz, Hahn & Holzgreve, 2007). Type1 immunity includes both the T helper -1 (Th1) axis which favors cellular cytotoxicity and promotion of innate immunity and secretion of type -1 cytokines (Interferon-γ (IFN-γ), IL-2, IL-12) from multiple other sources. Type 2 immunity is considered anti- inflammatory and includes both the T helper-2 (Th2) axis and secretion of type- 2 cytokines (IL-4, IL-5, and IL-13) from multiple other sources. The immune changes of pregnancy mimic in some sense how the immune system responds to chronic stress. Chronic stress induced immune dysregulation results in elevation of the inflammatory markers interleukin-6 (IL-6), IL-1β, tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP) (Coussons-Read, Okun & Nettles, 2007; Hansel, Hong, Camara & Kanel, 2010) which promotes latent herpes viral reactivation (Gesser, 1997; Miller & Cohen, 2005), increasing the risk of infection and behavioral and cognitive deficits (Godbout & Glaser, 2006; Haeri et al., 2011).

Psychosocial stress during pregnancy is a strong predictor of prenatal depression (Woods, Melville, Guo, Fan & Gavin, 2010; Escriba-Aguir, Royo-Marques, Artazcoz, Romito & Ruiz-Perez, 2012). Depression is one of the most common complications in pregnancy and as many as 20 percent of pregnant women experience it (Bonari, Pinto, Ahn, Einarson, Steiner & Koren,

2004; Groer et al., 2011). Prenatal depression increases the risks of developing postpartum depression. Prenatal depressive symptoms include sleep disturbance, lack of energy, and appetite changes. Depressive symptoms may associate with fatigue symptoms, for example Fagundes and colleagues (2012) reported that people with higher levels of depressive symptoms had greater fatigue symptoms. Fatigue symptoms include sleep disturbance, headache or musculoskeletal pain. These common symptoms may be unrecognized or misinterpreted as normal experiences of pregnancy (Marcus, 2009); however, unrecognized or untreated prenatal depression contributes to suicide, which is one of the leading causes of maternal death (Oates, 2003; Lindahl, Pearson & Colpe, 2004). Other negative pregnancy outcomes affected by depression include: preterm labor, low birth weight, and behavioral abnormalities in offspring (Buka, Cannon, Torrey & Yolken, 2007; Christian, 2012; Gennaro & Hennessy, 2003; Markham & Koenig, 2011; Schetter, 2011; Buka et al., 2001).

Herpes viruses have the ability to establish a long-term latent infection and to escape reactivation through host cellular immune surveillance. Latently infected cells may be stimulated during physiological or psychological stress to increase viral transcription, resulting in production of infectious virions which leads to either asymptomatic virus shedding or disease recurrence (Gesser, 1997).

Evidence suggests that psychosocial stress triggers immune system dysregulation which is associated with acute and chronic herpes infection (Miller & Cohen, 2005). Although important studies about stress and acute infection in humans that include the British common cold study (Cohen, Tyrrell & Smith, 1991) and the Pittsburgh influenza study (Cohen, Doyle & Skoner, 1999) have been conducted, they only examine general populations and fail to specify pregnant women as unique patients. Human studies about stress and chronic latent infectious disease

include oral herpes studies (Katcher et al., 1973), genital herpes studies (Goldmeier & Johnson, 1982), and ocular herpes studies have similar emphases. Very limited research has examined stress and immune dysregulation induced latent herpes viral reactivation in pregnant women.

Indoleamine-2, 3-dioxygenase (IDO) is an immunoregulatory enzyme which balances immunity and tolerance to reduce harm from pathogens that affects the biogenic amines pathway. IFN- γ and TNF-α have been shown in HeLa cells and astrocytoma cell cultures infected with herpes simplex virus type 2 (HSV-2) , to activate IDO which inhibit herpes simplex virus (HSV) replication (Adams, Besken, Oberdorfer, Mackenzie, Takikawa & Daubener, 2004). Stress dysregulation of the cellular immune response affects in both pathways, inducing tryptophan degradation in the IDO /tryptophan-2, 3-dioxygenase (TDO) pathway and activating phenylalanine to tyrosine enzymes in the guanosine-triphosphate-cyclohydrolase-1(GTP-CH1) pathway (Schrocksnadel et al., 2003; Capuron et al., 2011). T cells and natural killer (NK) cells secrete IFN-γ to induce IDO activity, which catalyzes tryptophan to break down to kynurenine, decreasing serotonin synthesis.

Although TDO follows the same pathway as IDO and both will break down tryptophan to kynurenine, this paper limits its focus to IDO. Enhanced neopterin and nitrite concentration, as well as decreased tyrosine levels, affect norepinephrine and dopamine synthesis, which is correlated to sleep disturbance, fatigue, gastrointestinal and muscular skeletal symptoms (Capuron et al., 2011; Schrocksnadel et al., 2003; Littrell, 2012; McTavish, Mannie, Harmer & Cowen, 2005; Scrandis et al., 2008; Widner, Laich, Spemer-Unterweger, Ledochowski & Fuchs, 2002). There is a lack of research findings on the relationship between IDO activity and GTP-CH1 in latent herpes viral reactivation and prenatal depression.

Statement of the Problem

In the general population, stress results in herpes viral reactivation. Psychosocial stress during pregnancy is a strong predictor of prenatal depression, and unrecognized prenatal depression contributes to suicide, which is the second most common cause of perinatal death (Oates, 2003; Lindahl, Pearson & Colpe, 2004). Prenatal depression needs to be addressed as it relates to herpes viral titers.

Purpose Statement

The purpose of this study is to identify the relationships among psychosocial stress, depression, herpes viral titers and biogenic amines in the second trimester of pregnancy. This study builds on *Influence of Lactation on Postpartum Stress and Immunity* (Grant number: R01NR005000).

Specific Aims

The specific aims are to analyze the relationships among stress, depressive symptoms, herpes viral titers, and metabolites of the IDO and GTP-CH1 pathways. The central hypothesis in this study is that prenatal psychosocial stress induces herpes viral reactivation through type-1 immune suppression and tryptophan degradation through the IDO pathway. The tryptophan degradation decreases serotonin synthesis which possibly accelerates depressive symptoms. It is hypothesized that stress favors reactivation of latent herpes viruses in pregnant women because they are vulnerable to stress. This hypothesis is formulated on the basis of data collected in the parent study (R01-NR005000) which investigated perinatal immune, endocrine and inflammatory changes in pregnancy and the postpartum period as well as reviews of the literature.

The current study examined previously assayed herpes viral: herpes simples virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), human herpes virus-6 (HHV-6) and cytomegalovirus (CMV) IgG titers from women at 16 to 25 gestational weeks and measured Epstein- Barr virus (EBV) antibody IgG titers in frozen plasma samples from these same women and the resulting information were added to the data base.

Hypotheses to be tested include:

Hypothesis 1: There is a significant positive relationship between stress and depressive symptoms in pregnant women.

Hypothesis 2: There are significant positive relationships among herpes viral titers, stress and depressive symptoms in pregnant women.

Hypothesis 3: The kynurenine /tryptophan ratios and phenylalanine /tyrosine ratios, and neopterin levels are positively related to herpes viral titers in depressed pregnant women.

Definitions of Relevant Terms

The relevant terms in this study will be referenced as following:

Stress. Stress is defined as "the circumstance, characteristics, and events which constitute, or are perceived to constitute, a threat to survival or well-being" (Walker, Green, Greenman, Walker & Sharp, 2005, p. 141). Hans Selye (1976) initially defined stress as a physical reaction to change as hypothalamic-pituitary-adrenal (HPA) and sympathoadrenomedullary (SAM) are co-activated and mediated in the immune system. Psychological stress can result from experience such as major life events, abuse, trauma, and environmental factors, and it is measured by the Perceived Stress Scale (PSS) in this study.

Latent viral reactivation and antibody titers. According to Miller and Cohen (2005) and Sinclair (2008), after primary infection, human herpes virus establishes a latent infection

under the control of a healthy immune surveillance. During latency, the virus maintains its genome without production of infectious virions. However, latent virus production ceases and does not aggravate illness symptoms in immune-competent individuals. This latent or clinically silent period can last for years or a lifetime.

Latent viral reactivation occurs under certain conditions, such as in immune-compromised individuals, and can result in serious diseases. Recent studies revealed that latent viral reactivation mechanism is linked to an epigenetic process that occurs after integrating genetic material into host cells (Weekes et al., 2013; Knipe et al., 2013; Liu, Wang, Yan, Zhang, Abecassis & Hummel, 2013; Woellmer & Hammerschmidt, 2013).

Latent herpes viruses can be reactivated and then begin replication at any time and trigger symptomatic clinical illness; for example, HSV-1 causes cold sores and encephalitis, HSV-2 causes genital lesions, HHV-6 causes encephalitis, hepatitis, and disseminated diseases, EBV causes infectious mononucleosis, Burkitt's lymphoma, Hodgkin's disease, and nasopharyngeal carcinoma, and CMV causes conjunctivitis and other conditions (Miller & Cohen, 2005; Murata & Tsurumi, 2013).

However, not all reactivated herpes viruses cause clinical illness even when there is evidence of reactivation. Operationally, latent viral reactivation is measured by viral antibody production. Elevation of antibody titers shows increasing viral replication; the high titers of herpes specific antibodies are an indication of ineffective cellular immune control of the virus.

Depression. Depression is a mood disturbance characterized by changes in mood, loss of interest or pleasure, cognitive function, sleep, appetite, and energy level ((Pratt & Brody, 2008). According to Diagnostic and Statistical Manual of Mental Disorder (DSM-IV, 2000) criteria, "depressive disorder is a period of at least two weeks of feeling sadness, hopelessness, and

discouragement. The individual should also experience at least four additional symptoms including: changes in appetite or weight, sleep and psychomotor activity, decreased energy, feels of worthlessness or guilt, difficulty thinking, concentrating or making decision, or recurrent thoughts of death or suicidal ideation or suicide attempt" (p.349). Depression in this study is measured by Profile of Mood States Depression /Dejection subscale (POMS-D). Refer to chapter 3 methods.

Significance to Nursing

Pregnant women make up a major vulnerable population for stress induced diseases. Stressful events, poor coping skills, lack of social support, and availability of resources can affect immune function which increases the susceptibility to infectious diseases and depressive episodes. Because of the relationship between psychosocial stress -induced latent herpes viral reactivation and depression, emotions affect infectious diseases progression which in turn complicates mental illness.

The biochemical and immune alterations of pregnancy may play roles in prenatal depression, which affects multiple pregnancy outcomes and are predictor of postpartum depression. This nursing research may lead to improved health care by integration of biological tool such as biomarkers with behavioral assessment. Prenatal depression needs to be addressed as a specific health problem in a vulnerable time in women's lives. The results will be significance in guiding health promotion and disease prevention during pregnancy.

CHAPTER TWO

REVIEW OF THE LITERATURE

This chapter reviews literature that is relevant to the study. Topics are focused on (1) overall relationships between stress, depression and infection; (2) body mass index and depression; (3) pregnant women's immune systems; (4) stress-induced immune dysregulation; (5) immune dysregulation and herpes viruses; (6) herpes viral reactivation; (7) neuroimmune pathways and depression; and (8) herpes viruses, indoleamine-2, 3-dioxygenase (IDO) activation and depression. The stress induced immune response inhibits herpes viral replication by increasing IDO activity and possibly accelerating depressive symptoms.

Conceptual/Theoretical Framework

The bio-behavioral framework of this study is presented in Figure 1. Prenatal psychosocial stress and the normal physiology of pregnancy induces a shift from a type 1 to a type 2 adaptive immune profile and a state of controlled inflammation, a profile which favors latent herpes viral reactivation. Inflammatory factors, high levels of cortisol, and IDO activation in pregnancy reduce available tryptophan which decreases the likelihood of viral replication and affects serotonin synthesis as well as phenylalanine and tyrosine metabolism.

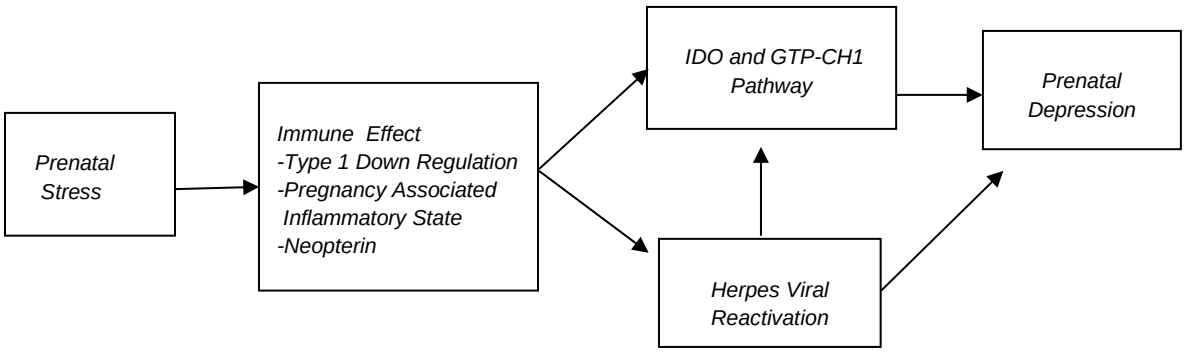


Figure 1. Relationship of prenatal stress, depression and herpes viral reactivation

Body Mass Index and Depression

The association between body mass index (BMI; kg /m^2) and depression in non-pregnant women has been previously reported (Onyike et al., 2003; Ma & Xiao, 2010). A recent study reported an association between obesity and suicidal ideation among US adult women (Zhao et al., 2012). Studies on the relationships between BMI and depression in pregnant women found inconsistent results.

LaCoursiere and colleagues (2006) reported that maternal BMI was related to postpartum depression studied in a large sample (N = 3,439). Bodnar and colleagues (2009) reported that pre- pregnancy obesity was associated with depression during pregnancy. LaCoursiere and colleagues in a later study (2010) (N = 1,053) also found that pre-pregnancy high BMI was related to postpartum depression. Contrary to LaCoursiere and colleagues (2006, 2010) and Bodnar (2009) studies, Haeri and colleagues (2011) reported lower BMI in a depressed pregnant women group (N = 100) than in non-depressed pregnant women group. Cassidy-Bushrow and colleagues (2012) also found that BMI did not mediate depressive symptoms in African-American pregnant women (N = 187).

Prenatal Stress, Depression and Infection

Psychosocial stress during pregnancy is a predictor of prenatal depression. Studies support a link between stress and depression in animal models (Markham & Koenig, 2011; Christian, 2012). However, limited data are available on relationships among stress, immune regulation, and depression in pregnant women. In maternal health there are limited studies with large samples or with prospective samples over 12 months of follow up. Recent studies by Woods and colleagues (2010) and Escriba-Aguir and colleagues (2012) showed common risk factors for maternal stress such as age, life events, low social support, maternal anxiety, and history of depression.

Prenatal depression occurs in up to 20 % of pregnancies and is influenced by hormonal, social, and genetic factors. Untreated prenatal depression contributes to postpartum depression (PPD), suicide events, preterm labor, and negative pregnancy outcomes (Oates, 2003; Lindahl, Pearson & Colpe, 2004; Bonari, Pinto, Ahn, Einarson, Steiner & Koren, 2004; Buka, Cannon, Torrey & Yolken, 2007; Marcus, 2009; Markham & Koenig, 2011). Prenatal depression is related to cortisol, dopamine, and serotonin monoamine neurotransmitters resulting in negative outcomes such as fetal growth delay, prematurity, and low birth weight (Field, Diego & Hernandez-Reif, 2010).

Evolutionary theories of depression developed from monoamine hypothesis, cytokine hypothesis, sickness behavior, and neuro-immune modulation to more recent "infection-defense hypothesis" or "pathogen host defense (PATHOS-D)" theory (Anders, Tanaka & Kinney, 2013; Raison & Miller, 2013). This "infection-defense hypothesis" states that depression is a behavioral response to help the immune system to fight with existing infection and to avoid additional exposure to pathogens. Increased exposure to infectious pathogens was associated

with increased rates of depression; the hypothesis is that risk of depression is due to individual immune system vulnerability to infectious pathogens or that individual carries depression risk candidate genes.

There are recent studies indicating that infections are associated with depression, for example, herpes simplex virus type 2 (HSV-2) infection was associated with depression in the general population (Pratt, Xu, McQuillan & Robitz, 2012), Epstein-Barr virus (EBV) and cytomegalovirus (CMV) reactivation were associated with inflammation and depression in older adults (Bennett et al., 2012). Depression in pregnant women was associated with influenza virus (Christian et al., 2010), EBV (Border et al., 2010; Haeri et al., 2011), human immunodeficiency virus (HIV) (Ross, Sawatphanit & Zeller, 2009) and *Toxoplasma gondii* infection (Groer et al., 2011). Additionally, in African American pregnant women depression was associated with EBV reactivation (Christian, Iams, Porter & Glaser, 2012; Haeri et al., 2011).

Immune Regulation in Normal Pregnancy

During pregnancy, the immune system plays an important role in avoiding rejection of the fetus. The theory of pregnancy immune tolerance views the fetus as a semi-allogeneic graft. There is reduced fetal trophoblastic cell membrane human leukocyte antigens (HLA) expression (Chen, Liu & Sytwn, 2012; Rusterholz, Hahn & Holzgreve, 2007). In addition, soluble factors from trophoblastic cells, such as IDO, inhibit maternal T cell proliferation and decrease natural killer (NK) cytotoxicity and interferon- γ (IFN-γ) production to avoid rejection of the fetus in early pregnancy (Chen, Liu & Sytwn 2012; Rusterholz, Hahn & Holzgreve, 2007).

During the first and third trimesters there is an up -regulated inflammatory state (Mor & Cardenas, 2010) as well as expression of cytotoxic T lymphocyte antigen (CTLA)-4 on T regulatory cells to induce monocyte and dendritic cell IDO activation, which is required to

maintain immunologic homeostasis during pregnancy (Chen, Liu & Sytwn, 2012). Because this process leads the placenta to secrete IDO, and IDO steals the essential amino acid tryptophan from T cells, it can result in decrease levels of serotonin, which is linked to depression.

Stress-Induced Immune Dysregulation

Miller, Maletic and Raison (2009) pointed out that many studies that recognized depression in non-pregnant individuals were associated with an inflammatory process that may be induced when stress stimulates the inflammatory transcription factor, nuclear factor kappa B (NF- κ B), through the sympathetic nervous system. It has also been found that stress increases glucocorticoid and catecholamine production, suppresses neutrophil and NK activity, elevates inflammatory cytokines, and shifts immunity to a type -2 cytokine profile. This immune response during a stressful event could change neurotransmitter balance, resulting in vulnerability to depressive symptoms and herpes viral reactivation (Black, 1994; Borders et al., 2010; Groer, Meagher & Kendall-Tackett, 2010; Padgett & Glaser, 2003; Segerstrom & Miller, 2004; Steptoe, Hamer & Chida, 2007; Haroon, Raison & Miller, 2012; Littrell, 2011; Miller, Maletic & Raison, 2009; Reiche, Nunes & Morimoto, 2004; Messay, Lim & Marsland, 2012; Bao, Meynen & Swaab, 2008; Christian, 2012; Hansel, Hong, Camara & Kanel, 2010; Godbout & Glaser, 2006).

Whether these same phenomena occur in pregnancy is largely unknown, as only a few studies with small sample sizes have been reported. Coussons-Read and colleagues (2005, 2007) reported that significantly high levels of perceived stress during the first and third trimester of pregnancy (N = 24) resulted in increased circulating levels of C-reactive protein (CRP) and pro-inflammatory cytokines interleukin-1β (IL-1β), IL-6, and tumor necrosis factor- α (TNF-α), and decreased levels of the anti-inflammatory cytokine IL-10. Christian and colleagues (2009) reported in a sample size of 60 that depressive symptoms were significantly associated with IL-6,

TNF-α at 15 weeks of gestation. Conflicting data was reported from Blackmore and colleagues (2011), as they did not find a relationship between depression and pro-inflammatory cytokines in pregnant women.

Immune Dysregulation and Herpes Viruses

The stress-induced immune shift of cytokines from the Th1 to the Th2 profile favors virus reactivation. For example, psychosocial stress was associated with increased IL-6 and CRP levels which prompted depression or latent virus reactivation in the general population (Bennett et al., 2012; Gouin, Glaser, Malarkey & Beversdorf, 2012; Fagundes, Glaser, Hwang, Malarkey & Kiecolt-Glaser, 2013). Hypotheses on the impact of stress on viral reactivation began in the early 1970s. Gesser (1997) reported that there was potential to synthesize fully infectious viruses under permissive conditions during the latency stage of herpes viral infection. Latently infected cells may be provoked by physiological or psychological stimuli, hypothalamic-pituitary-adrenal (HPA), and glucocorticoid hormones to produce infectious virions, which can lead to either asymptomatic virus shedding or disease recurrence (Pereira et al., 2003).

Evidence from human studies suggests that stressful experiences increase vulnerability to viral infections including chronic herpes viral reactivation (Cohen, Tyrrell & Smith, 1991; Katcher, Brightman, Luborsky & Ship, 1973; Goldmeier & Johnson, 1982; Cohen, Doyle & Skoner, 1999; Glaser et al., 1999; Chida & Mao, 2009). Glaser and colleagues (1999) observed a relationship between psychological stressor and herpes viral (HSV-1, HHV-6, EBV) reactivation. Although Phillips and colleagues (2008) conducted a study of the relationship between older adults' CMV antibodies titers and depressive symptoms, it design did not adequate measure stress and latent herpes viral reactivation.

The possibility that physical and psychosocial stress can induce immune dysregulation and latent viral reactivation in pregnancy is largely unknown and no studies have examined the effect of stress, depressive symptoms and herpes viral titers in pregnant women. A few studies reported that perceived stress was associated with pro-inflammatory and anti-inflammatory cytokines in pregnant women; Christian and colleagues (2009) reported that immune alteration has a negative effect on response to infectious pathogens during pregnancy.

Two recent studies reported that chronic stress induced EBV antibody titer elevation is associated with maternal depression (Borders et al., 2010; Haeri et al., 2011). These data provide evidence of a link between maternal stress, depression, and viral reactivation; however, further studies are needed to evaluate the bio-behavioral pathways.

Herpes Viral Reactivation

Herpesviridae is a double-stranded DNA enveloped virus. Herpes virus family members (HSV-1, HSV-2, CMV, HHV-6, and EBV) are a leading cause of human viral diseases. These herpes viruses are infecting in the human central nervous system. After infection, viruses replicate and integrate their DNA into the host genome. Once integrating, viruses establish long-term latent infection and are capable of escaping host cell immune surveillance.

There is more evidence showing that neurotropic potential herpes viruses are related to psychiatric disorders; for example, Dickerson and colleagues (2003) found that HSV-1 seropositivity is an independent predictor of cognitive dysfunction in schizophrenia patients. Buka and colleagues (2007) reported that mothers with HSV-2 infection were at significantly increased risk of their offspring developing psychosis. CMV and EBV are related to stress, anxiety, depression and fatigue syndrome in elderly or cancer patients (Fagundes et al., 2012; Bennett et al., 2011). Recently, Carter (2013) reported that maternal HSV-1 infection could

affect fetal neurodevelopment or adult psychiatric and neurological disorders through a host/pathogen interactome and susceptibility genes interaction, resulting in direct or collateral immune and inflammatory effects.

Herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2). Herpes simplex virus is an alpha-herpesvirinae. The HSV-1 infection is often found in orofacial lesions or trigeminal ganglia. HSV-2 commonly causes genital herpes. Following primary infection of neurotropic herpes simplex virus, the virus develops a latent infection in neuronal nuclei within sensory ganglia. Most individuals are asymptomatic after the latent virus remains in the sensory neurons. Effective control of herpes virus is mediated by T cells rather than antibodies. High titers of herpes-specific antibodies indicate increased viral replication and ineffective cellular immune control of virus.

In vitro and animal studies showed that immune system responses that involve IFN-γ production and HSV specific memory and effector CD8+ T cells play a key role in regulating latent herpes virus expression and replication (Freeman, Sheridan, Bonneau & Hendricks, 2007; Khanna, Lepisto, Decman & Hendricks, 2004; Huang, Xie & Xu, 2011). Herpes viral latency immunity is dependent on HSV-specific CD8+ T cells production of IFN-γ to block HSV-1 reactivation.

Additionally, Freeman and colleagues (2007) in their animal study showed that stress led to a significant reduction of HSV-1 specific CD8+ T cells IFN-γ production and decreased the number of CD8+ T cells around the nerve ganglion in response to virus reactivation. Latent virus reactivation results in virus leakage from latency, anterograde transport to the periphery and releases virions at the skin or mucosal surface (Pereira et al., 2003).

Goldmeier and colleagues (2008) suggested that chronic stress could lead to increased rates of recurrence of the herpes viral infection. Sainz and colleagues (2001) proposed that stress compromises individual cellular immune responses, influences the herpes virus latency-associated transcript gene (LAT) expression and triggers HSV reactivation. Pereira and colleagues (2003) examined the relationship between stress and genital herpes recurrence in African American women (N = 34) with the HIV infection during the one year follow-up. They found that life stress was highly predictive of genital herpes recurrence after controlling for HIV and the relevant behavioral factors of smoking and protease inhibitor usage. Cruess and colleagues (2000) reported that HIV positive men had a lower stress score and lower HSV-2 IgG titers after ten weeks of stress relaxation intervention.

Human herpes virus-6 (HHV-6). HHV-6, a beta-herpesvirinae, is the most common cause of childhood diseases - Roseola infantum or Exanthema infection. When first infected with HHV-6, the virus replicates in the salivary glands and secretes in saliva. After primary infection, the virus remains latent in the lymphocytes and monocytes and is generally asymptomatic in healthy individuals. Some cases reported that HHV-6 was associated with central nervous system diseases such as seizure, multiple sclerosis or chronic fatigue syndromes. Activated placental HHV-6 infection during pregnancy was associated with congenital HHV-6 infection (Caserta et al., 2007; Campadelli-Fiume, Mirandola & Menotti, 1999).

Cytomegalovirus (CMV). CMV is a beta-herpesvirinae and infection rates are as high as 90 % in the population. CMV infection, similar to other herpes viruses, can be primary or latently reactivated. Transmission of CMV occurs through infected person's shedding CMV in various body fluids, such as blood, urine, semen, breast milk and saliva. Immunocompetent women infected with CMV are usually asymptomatic. Symptoms reported are generally vague

with fever, myalgia, and malaise. CMV is capable of establishing lifelong latent infection in epithelial, endothelial, smooth muscle and connective tissue cells under the control of a healthy immune system. Individuals with immunosuppression or organ transplantation are at increased risk of CMV infection or reactivation Congenital CMV infection occurs from the placenta to the uterus which causes neonates deafness, blindness, mental retardation or death (Johnson & Anderson, 2013; Liu et al., 2013).

CMV has been indicated as a cell-mediated immune function marker and is linked to stress. Stress down- regulated cellular immunity and increased inflammatory cytokines TNF-α and IL-6 permit viral reactivation which leads to increased CMV-specific IgG levels (Dowd, Aiello, Chyu, Huang & McDade, 2011). Phillips and colleagues (2008) conducted a study of the relationship between CMV antibodies titers, depression, and anxiety in older adults. Results found that higher CMV antibody titer was associated with mood disorders.

Recently, Liu and colleagues (2013) proposed an epigenetic model of CMV latent reactivation. The model suggests that inflammatory cytokines and oxidative stress activate transcription factors to bind to the major immediate early promoter (MIEP) gene and induce chromatin remodeling to activate viral immediate early gene (IE gene) expression. This IE gene does not reactivate and produce infectious viruses in an immunocompetent person, but it does reactivate in an immunosuppressed person.

Epstein -Barr virus (EBV). EBV is a gamma-herpesvirinae which transmitted through oral secretions. The virus remains in the host through life and is carried in a latent form in peripheral blood B lymphocytes. EBV expresses its viral genome in newly divided or resting B cells to increase the reservoir of latent virus. EBV latent proteins include six nuclear antigens (EBNAs 1, 2, 3a, 3b, 3c and LP) and three latent membrane proteins (LMP1, 2A and 2B).

EBNA, early antigen (EA), viral capsid antigen (VCA), and latent membrane protein are the major virus antigens. EBV EBNA1 is the only viral protein needed to maintain latency in the circulation. EBV VCA and anti-EBNA immunoglobulin G (IgG) are always present in the seropositive individual (Gesser, 1997; Coskun et al., 2010).

After primary infection, EBV remains in its latent form in healthy individuals and undergoes sporadic reactivation. Over 90 % of the adult population has EBV latent infection, and evidence suggests that EBV reactivation is correlated with stress-induced immune dysregulation in response to infection. Immune dysregulation induces EBV replication which leads to increased EA and VCA IgG antibody production (Coskun et al., 2010; Christian, 2011).

Maternal EBV reactivation causes early fetal death, early delivery, and low birth weight infants. Latent EBV infection has been linked to acute lymphoblastic leukemia in infected mothers' offspring (Eskild, Bruu, Stray-Pedersen & Jenum, 2005).

Glaser and colleagues (1999) conducted a study to observe the relationship between psychological stressor and herpes viral reactivation by measuring HSV-1, HHV-6 and EBV VCA IgG antibodies titer in military academy students during and after a six-week training period. Significant elevation of EBV antibody titers was associated with a diversity of psychological stressors. This study provided the base research model in stress and latent herpes viral reactivation.

Neuroimmune Pathways and Depression

Research has shown that depression is often associated with imbalance in monoamine neurotransmitters metabolism (Sperner-Unterweger, Kohl & Fuchs, 2012; Felger et al., 2012; Haroon, Raison & Miller, 2012; Littrell, 2012; Capuron & Miller, 2011; Capuron et al., 2011; McTavish, Mannie, Harmer & Cowen, 2005; Scrandis et al., 2008, Widner, Laich, Sperner-

Unterweger, Ledochowski & Fuchs, 2002). Inflammatory factors such as cytokines and reactive oxygen species (ROS) drive biogenic amines in such a way that dopamine, melatonin and serotonin are decreased, and these neurotransmitters all play important roles in the pathophysiology of depression. Figure 2 shows the biogenic amine pathways affected by inflammation.

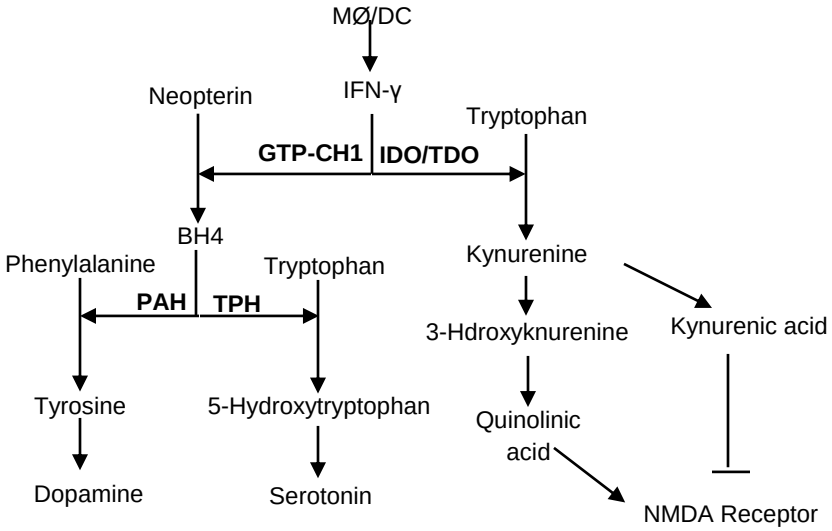


Figure 2. Biogenic amine pathways affected by inflammation
MØ: macrophage, DC: dendritic cells, PAH: phenylalanine-hydroxylase, TPH: tryptophan hydroxylase, GTP-CH1: guanosine-triphosphate-cyclohydrolase-1, IDO: indoleamine-2, 3-dioxygenase, TDO: tryptophan-2, 3-dioxygenase, BH4: tetrahydrobiopterin, NMDA: N-methyl-D-aspartate

In pregnancy, while type 1 adaptive immunity is somewhat suppressed, the innate immune system is highly activated. The pathway may thus be altered in pregnancy in the following ways: (1) high levels of corticosteroids activate tryptophan-2, 3-dioxygenase (TDO), which is the first step in the conversion of tryptophan to kynurenine, (2) inflammatory cytokines induce IDO catabolism of tryptophan into kynurenine; both TDO and IDO activation reduces serotonin synthesis, (3) inflammatory cytokines and reactive oxygen species (ROS) decrease 5,6,7,8-tetrahydrobiopterin (BH4) and increase neopterin levels through activation of GTP-CH1 activity, (4) reduction of BH4 (hydroxylase cofactor) leads to impairment in the conversion of

phenylalanine to tyrosine, tyrosine to dopamine, dopamine to norepinephrine, epinephrine and tryptophan to serotonin. These relationships are depicted in Figure 2**.** Capuron and colleagues (2011) reported that serotonin and dopamine metabolic pathways were associated with depressive symptoms in the elderly. Currently no studies were found that examined IDO /TDO and GTP-CH1 pathways in prenatal depression.

Neopterin, nitrite and BH4. Neopterin is secreted by monocytes or macrophages upon activation by IFN-γ from T cells and is augmented by ROS, nitrite is the end product of nitric oxide (NO) and plays a role in immune process and metabolic regulation and BH4 is a cofactor of phenylalanine-, tyrosine- and tryptophan-5-hydroxylase and NO synthases. The function of neopterin is not well understood, but it is believed to possibly induce nuclear factor- kappa B (NF- *k* B) expression and trigger apoptosis. Several studies have measured neopterin, together with tryptophan and kynurenine level, to assess the relationship between immune activation and depression (Widner, Laich, Sperner-Unterweger, Ledochowski & Fuchs, 2002; Berdowska & Zwirska-Korczala 2001; von Versen-Hoeynelk et al., 2009; Murr, Widner, Wirleitner & Fuchs, 2002).

Tryptophan, IDO and kynurenine. Tryptophan is important for serotonin and melatonin biosynthesis. Tryptophan catabolizes through two pathways. One is through tryptophan 5-hydroxylase decarboxylation to form serotonin. The other pathway is the kynurenine pathway through TDO and IDO, in which tryptophan is catabolized to kynurenine, leading to degradation into the 3-hydroxykynurenine, quinolinic acid, and kynurenic acid pathway. Kynurenic acid crosses the blood-brain barrier and acts on the glutaminergic receptor, N-methyl-D-aspartate (NMDA), which is associated with the functions of cognitive memory, learning, and attention (Haroon, Raison & Miller, 2012; Dantzer, O'Connor, Freund, Johnson & Kelley, 2008;

Schrocksnadel, Wirleitner, Winkler & Fucs, 2006; Widner et al., 2002; Kurz et al., 2012; Capuron et al., 2011; Sublette & Postolache, 2012; Miller, Maletic & Raison, 2009).

Depressive symptoms have been linked to cytokines IFN-γ and IL-2 induced IDO activation in recent studies. O'Connor and colleagues (2009) investigated relationships between depressive symptoms, IDO activation, and tryptophan depletion in an animal study by injecting Bacilli Calmette-Guerin (BCG) in mice. In 2012, Elovainio and colleagues studied the relationship between IDO activation and depressive symptoms in a large population of healthy young Finns. The study found that IDO activity was a predictor for future depression development in women.

In addition to the inflammatory pathways described above, pregnant women have lower tryptophan due to the placenta producing large amounts of IDO to achieve immune tolerance for the fetus. Tryptophan is an essential amino acid required by T cells, and activation of IDO starves T cells that could potentially react to fetal antigens; however, decreased tryptophan would also reduce serotonin synthesis and potentially increase the risk of depression (Munn et al., 1998; Kohl et al., 2005). IDO activity can be assessed by ratio of kynurenine to tryptophan.

Two studies provide evidence of IDO activation and low tryptophan during normal pregnancy (Schrocksnadel et al., 2003; Alegra, Diaz-Lagares & Gonzalwz, 2008). Additionally, Scrandis and colleagues (2008) conducted a small sample (N = 27) longitudinal study to examine CRP elevation, IDO activation, and tryptophan depletion in women with prenatal depressive symptoms. They concluded that CRP and IL-6 elevation were correlated with IDO pathway tryptophan depletion and kynurenine elevation which were associated with prenatal and postpartum depression. Research is needed to examine the relationship between IDO activation and depression in pregnant women.

Phenylalanine and tyrosine. BH4 is an important cofactor of several hydroxylases, including phenylalanine-hydroxylase (PAH). Phenylalanine is catalyzed by PAH to produce tyrosine, a precursor for dopamine biosynthesis (Sperner-Unterweger, Kohl & Fuchs, 2012; Capuron et al., 2011; Ploder, Neurauter, Spittler, Schrocksnadel, Roth & Fuchs, 2007; McTavish, Mannie, Harmer & Cowen, 2005; Leyto et al., 1999). An increase in either serum phenylalanine concentration or phenylalanine to tyrosine ratio (Phe /Tyr) may be due to decreased PAH activity, resulting from immune activation and inflammation that inhibits BH4 activity. There is evidence of increased phenylalanine levels in the blood correlated to trauma, sepsis, viral infection, and cancer (Ploder et al., 2005; Neurauter et al., 2008; Zangerle et al., 2010), or tyrosine dietary depletion (McTavish, Mannie, Harmer & Cowen, 2005; Parker & Brotchie, 2011). Capuron and colleagues (2011) found BH4 deficiency and increased blood neopterin in depression and schizophrenia patients.

Herpes Viruses, IDO Activation and Depression

Effective control of herpes virus is mediated by cytotoxic T cells rather than antibodies. High titers of herpes-specific antibodies are an indication of increased viral replication and ineffective cellular immune control over the virus. Recent in vitro and animal studies by Huang (2011), Freeman (2007), and Khanna (2004) showed that IFN-γ production and HSV specific memory and effector CD8+ T cells play a key role in regulating latent herpes viral expression and replication. Immune activation of IDO decreases tryptophan concentration, slowing down protein synthesis to inhibit pathogens or malignant cell growth. Chronic infection leads to increased IFN levels which affect serotonin synthesis and glutamate and quinolinic acid levels causing depression (Haroon et al., 2012; Schrocksnadel, Wirleitner, Winkler & Fuchs, 2006).

Korf and colleagues (2002) referred to studies on depression and herpes viral reactivation as consequence of activation of the inflammatory response system which is mediated by the limbic system. Limited research has shown the connection between IDO activation, herpes virus and depression; however, Bellman-Weiler and colleagues (2008) showed fatigue symptoms in chronic EBV infection, HIV, and cancer patients are associated with herpes viral titers (Bellman-Weiler et al., 2008; Fagundes et al., 2012). This study reported that patients with detectable EBV DNA and IgA titers had higher neopterin, IFN-γ mediated IDO activity and lower tryptophan concentration, which correlated to fatigue symptoms.

Adams and colleagues (2004) reported that IFN-γ and TNF-α induced IDO activity, which inhibited HSV replication. Conflicting data reported by Divanovic and colleagues (2012) showed that there was no evident role of IDO in HSV-1 infection. Whether the low level of tryptophan is due to IDO activation or not is not fully understood. The new 5-HT (5-hydroxytrptamine) hypothesis of depression, which states that cellular immune activation induces IDO activation which disrupts tryptophan metabolism to affect serotonin synthesis, is currently under examination (Maes, Leonard, Myint, Kubera & Verkerk, 2011; Sublette et al., 2011).

Chapter Summary

Pregnancy is a vulnerable time for women due to physiological and psychosocial changes. Research shows that there is a positive relationship between prenatal psychosocial stress and depression. A growing number of studies support the hypothesis that elevation of pro-inflammatory cytokines is associated with depressive symptoms in pregnant women; however, there are still some conflicting results about the relationship between depressive symptoms and inflammatory cytokines in pregnant women.

There is very little known about the role of inflammation or pregnancy induced IDO and GTP-CH1 activation resulting in alternated biogenic amines in prenatal depression. Further research is needed regarding psychosocial stress, latent viral reactivation, and biogenic amines metabolites in prenatal depression or depressive symptoms.

CHAPTER THREE

METHODS

This chapter focused on the study design, sample, and setting. The measures used in the secondary data were analyzed to measure EBV VCA IgG titer.

Research Design, Sample and Setting

Parent R01 study method. This exploratory study consists of a secondary analysis of the Perceived Stress Scale (PSS), the Profile of Mood States (POMS), biogenic amines, herpes viral titers, and EBV antibody IgG titer from stored plasma samples from pregnant women enrolled in a funded study (R01NR05000). The study participants were recruited from University of South Florida (USF), Health Department and Tampa General Hospital Obstetrical and Gynecology practice sites. The target population was focused on pregnant women between the ages of 18 and 45 years at 16 to 25 gestational weeks. A total sample of 631 pregnant women was recruited in this original study from 2007-2011. Data from a sample of 380 pregnant women was available to analyze in the current study.

Inclusion criteria for the study were pregnant women between 18 to 45 years and all minority groups. Exclusion criteria were women with HIV/AIDS, those with immune disease, people taking immune altering medications, participants who were in vitro fertilized, illegal substance abusers, and extremely thin women. This study was feasible because of the large sample size and availability of a well-established bio-behavioral laboratory to perform assays at the USF College of Nursing.

Measures

Demographic information, two psychological questionnaires (PSS and POMS) and 15 mL of blood were collected from each participant during the prenatal time of enrollment between 16 to 25 weeks of gestation. This plasma was analyzed for a panel of herpes viral titers: HSV-1, HSV-2, HHV-6, CMV, thyroid peroxidase antibody (TPO) and *Toxoplasma gondii* antibody in the parent study in Dr. Robert Yolken's laboratory at Johns Hopkins School of Medicine. In addition a panel of biogenic amines: nitrite, tryptophan, kynurenine, phenylalanine, and tyrosine were measured by high-performance liquid chromatography (HPLC) in Dr. Dietmar Fuchs's laboratory at the University of Salzburg, Austria. Kynurenine /tryptophan (KYN /TRP) ratios phenylalanine /tyrosine (Phe/Tyr) ratios were calculated and neopterin was measured by enzyme-linked immunosorbent assay (ELISA). The investigator added to the viral panel by assaying EBV antibody IgG titer by ELISA. The variables in this study are presented in Table 1.

Demographic data. Baseline data, which were collected at the time of recruitment, included age, ethnicity, years of education, income, gravidity, the presence of any chronic or acute health problems, current medications, and height and weight at the time of enrollment to calculate body mass index (BMI).

Perceived stress scale (PSS). The PSS is a 14-item instrument to evaluate cognitions and emotions related to perceived general stress that an individual felt or thought of as stressful in the week preceding the measurement. Participants completed the PSS at the time of enrollment in 16 to 25 gestational weeks. The PSS-14 uses a 5-point Likert-type summated rating scale response format (0 = never to 4 = very often) and has a range of 0 to 56. The PSS consistently has had Cronbach's alphas between .84 and .86 (Cohen, Kamarck & Mermelstein, 1983). The PSS also has short-term test-retest reliability (.85) as well as constructed validity. The parent R01 pregnant

and postpartum thyroid peroxidase antibody study had PSS Cronbach's alpha (α) = .84 (Groer & Vaughn, 2013).

Table 1
Table of Variables from Parent R01 and Current Study

Variable	Measure- Instrument	Measurement
R01 Study		
Demographics	Age, ethnicity, BMI, Number of children, Number of pregnancy	Self-report
Stress	Perceived Stress Scale (PSS)	Self-report
Depressive Symptoms	Profile of Mood States-Depression Scale	Self-report
Fatigue Symptoms	Profile of Mood States-Fatigue Scale	Self-report
Herpes Virus Titers	HSV-1, HSV-2, HHV-6, CMV	ELISA
Biogenic Amines	Tryptophan, Kynurenine, Kynurenine/Tryptophan ratio, Phenylalanine, Tyrosine, Phenylalanine/Tyrosine ratio, Nitrite	HPLC ELISA
Inflammatory Protein	Neopterin	ELISA
Other Biomarker	*Toxoplasma gondii* IgG	ELISA
	TPO Antibody	ELISA
Current Study		
Herpes Virus Titers	EBV VCA-IgG	ELISA

Note: ELISA: Enzyme-linked immunosorbent assay, HPLC: High-performance liquid chromatography, HSV: Herpes simplex virus, HHV-6: Human herpes virus-6, CMV: Cytomegalovirus, TPO: Thyroid peroxidase, EBV: Epstein-Barr virus, VCA: Viral capsid antigen

Profile of mood states (POMS). Participants completed the POMS (McNair, Lorr & Droppleman, 1992) at the time of enrollment. The instrument provided a multi-dimensional assessment of mood to measure how the participant had felt over the week prior to enrollment. The POMS is a 65-item, 5-point Likert-type scale (0 = not at all to 4 = extremely) and has a total mood disturbance score and 6 subscales: depression-dejection (15 items, score range 0 -60), tension-anxiety (9 items, score range 0 -36), confusion-bewilderment (7 items, score range 0-

28), fatigue-inertia (7 items, score range 0 -28), anger-hostility (12 items, score range 0 - 48), and vigor-activity (8 items, score range 0 -28).

POMS-Fatigue (POMS-F) score is determined by calculation the scores for worn out, listless, fatigued, exhausted, sluggish, weary and bushed. The POMS depression-dejection score (POMS-D) is determined by calculation the scores for unhappy, sorry for things done, sad, blue, hopeless, unworthy, discouraged, lonely, miserable, gloomy, desperate, helpless, worthless, terrified and guilty. The POMS-F scores range from 0 – 28 and POMS-D scores range from 0 to 60, with higher scores suggesting increased mood disturbance during the past week.

Many studies reported POMS has good internal consistency, reliability, and moderate test-retest reliability (Griffith et al., 2005). POMS scores have internal consistency from .87 to .92 and test-retest reliabilities ranging from .65 to .74 (Cruess et al., 2000). The parent R01 study pregnant and postpartum thyroid peroxidase antibody study had POMS-D Cronbach's alpha (α) = .91 (Groer & Vaughn, 2013). The POMS-D cut-off score for clinical depression screening is 20. Participants with a POMS-D score over 20 were referred to healthcare providers for further evaluation of depressive symptoms.

Blood collection and processing. When participants between 16 to 25 weeks of pregnancy enrolled, their peripheral blood samples (15 mL) were collected by antecubital venipuncture, kept cold, and brought to the bio-behavioral laboratory within two hours. The blood was centrifuged at 3800 rpm for 25 minutes at 4°C. The plasma supernatants were aliquotted and stored at -80° C. After samples were collected, they were analyzed by herpes viral IgG immunoassay and biogenic amines chromatography assays.

Herpes viral IgG immunoassay. HSV-1, HSV-2, HHV-6, and CMV IgG and *Toxoplasma gondii* IgG serology performed by solid-phase enzyme immunoassay method at The Stanley

Virology laboratory at Johns Hopkins University by Dr. Robert Yolken. EBV antibody IgG serology followed by manufacturer (ALPCO) protocol and was performed at the bio-behavioral laboratory at College of Nursing, University of South Florida.

Biogenic amines chromatography. Plasma tryptophan (μmol/L), kynurenine (μmol/L), phenylalanine (μmol/L) and tyrosine (μmol/L) were analyzed by high-performance liquid chromatography (Winder, Werner, Schennach, Wachter & Fuchs, 1999) by Dr. Dietmar Fuchs at the University of Innsbruck, Austria. The kynurenine to tryptophan and phenylalanine to tyrosine ratios were calculated by this method. Neopterin (μmol/L) and nitrite (μmol/L) were measured by ELISA (BRAHMS, Hennigsdorf, Germany) with detection limits of 2 nmol/L.

Procedures

The parent study was approved clinic sites and the University of South Florida (USF) Institutional Review Board (IRB) prior to the collection of data. Pregnant women between 16 to 25 weeks' gestation who met inclusion criteria were recruited from USF's Obstetrical and Gynecology clinic, Tampa General Hospital Genesis clinic and the Health Department in Tampa.

The participants were informed that there was a possibility that the researchers would discover thyroid problems during the course of the study and that the participants would be referred back to their providers for diagnosis and treatment. They gave permission for the researchers to contact their health providers if any significant health problems were to be discovered during the course of the study. This would include both physical and mental health problems. All pregnant women were assured of the confidentiality of all data. Participants were provided time to read the consent document, discuss the information, and ask questions. Participants then signed the informed consent for the study.

The research data were obtained from demographic data, psychosocial self-report questionnaires, and blood samples (12-15 mL) in clinic offices during one of the participant's prenatal routine appointment. The blood was sent to the bio-behavior laboratory at College of Nursing for analysis related to the study. Plasma samples were stored in laboratory freezers at -80° C. Biohazard procedures were followed in the performance of the assay and the disposal of wastes.

EBV VCA IgG immunoassay. EBV viral capsid antigen (VCA) IgG ELISA assay (ALPCO, 20-EVGHU-E01, 96 wells commercial kit) that measures EBV VCA IgG was used to determine EBV titer and all analyses were performed by the PhD candidate. All ALPCO reagents, ELISA plates and plasma samples were allowed to reach room temperature before use on the day of assay.

First, the wash solution was warmed to 37° C and crystals were completely dissolved before use. Diluted wash solution was prepared by adding 30 mL buffer solution to 570 mL fresh distilled water. Plasma aliquots kept at -80° C were thawed at room temperature and centrifuged (5430 R eppendorf) 5000 revolutions per minutes (rpm) for 5 minutes before the assay. Plasma samples were diluted 1:101 with a sample diluent buffer according to the protocol provided by manufacture, mixed well by vortex (Fixed Speed Mini Vortexer, Fish Scientific) and left at 15 minutes before dispensing to 96 wells.

Each ELISA plate was run using duplicate wells for blank background, three controls (negative control, cut-off control, and positive control), and diluted samples. Wells were covered with foil after 100 µL were dispensed into each well and incubated for 60 minutes at 37° C incubator. Following incubation, a plate washer (Microplate washer, Finstruments) was used to rinse the wells five times with diluted wash solution.

After rinsing the plate, 100 μL Enzyme Conjugate Solution were dispensed into each well except blank control wells, and then the plate was incubated in the dark, at room temperature for 30 minutes. Following incubation, a plate washer was used to rinse the wells five times with diluted wash solution. Residual droplets were removed and then 100 μL of Substrate Solution were added into all wells. The plate was incubated in the dark, at room temperature for exactly 15 minutes. After incubation, Stop Solution was added to each well to stop enzymatic reaction. After adding the Stop Solution, the plate was placed into microtiter plate reader (Thermo Labsystems Multiskan MCC/340, Fisher Scientific) and read at the optical density (OD) of 450/620 nm.

EBV IgG titers calculation. EBV IgG titer was calculated by the mean absorbance values at the OD 450 nm of all duplicates wells minus blank background. A final result in units (DU) was calculated by sample (mean) absorbance value multiplied by 10 and divided by the cut off control (CO).

Storage of study materials. The data were coded and remained confidential. Study forms were stored in a locked file cabinet at the University, and only the Principle Investigator (PI) and study personnel have access to these forms. Electronic data files were de-identified, and files were password protected. The proposed procedures of this secondary analysis were reviewed by the University IRB prior to the initiation of the study. This candidate's addendum to the current research protocol has been approved by University of South Florida IRB (Ame3_104998) on 3/21/2013 (See Appendix D). The original forms would not be accessed for the purposes of this secondary data analysis. De-identified study data files were obtained from R01 study Principle Investigator, Dr. Groer.

Data Analysis Plan

Preliminary data analyses. Using Microsoft Excel software version 2010 and Statistical Package for Social Sciences (SPSS) computer software version 21, data cleaning and data processing were performed to detect corrupt or inaccurate data.

Univariate data analysis in this study included descriptive statistics, means and standard deviations of all the variables with perceived stress scores, depression scores, biogenic amines, and herpes viral IgG titers. Histograms, scatterplots, and line graphs were used to examine the data. All of the data were analyzed for normality, outliers, and missing data. Common logarithm (log10) transformations were performed if needed for negative skewness and outliers were checked. Covariates were analyzed included age, race, number of children, BMI, *Toxoplasma gondii* antibody and thyroid peroxidase antibody (TPO) titers.

Bivariate data analysis in this study included: (1) Chi-square tests for two categorical variables, (2) independent sample t-tests to compare two different groups mean scores, and (3) Pearson's product moment correlations to assess the relations between continuous variables and depressive symptoms scores.

Correlations among variables were calculated using Pearson's product moment correlation coefficients (r) to evaluate the association between stress, depression scores, POMS other subscales, tryptophan, kynurenine, phenylalanine, tyrosine, neopterin, nitrite, herpes viral IgG titers (HSV-1, HSV-2, HHV-6, CMV, EBV) and also ratios of kynurenine to tryptophan and phenylalanine to tyrosine. The correlation coefficient is a measure of linear association between two variables. A correlation coefficient value is between -1 and +1. A value of +1 indicates that two variables are perfectly related, a value of -1 indicates a perfectly negative linear relationship, and 0 indicates that there is no linear relationship between two variables (Hays, 1994).

Multivariate data analysis in this study included one-way analysis of variance (ANOVA) to compare mean scores of two or more groups and multiple regression analysis to explore the relationship between one continuous dependent variable (e.g. perceived stress, depressive symptoms) and a number of continuous independent variables.

In multiple regression analysis, assuming a relationship between the independent and dependent variable, the probability of making a type I error to falsely reject a correct model is .05 at a statistical power level of .8. The required sample size for regression is $N \geq 50+8$ m (m is the number of independent variables) for testing the multiple regression and $N \geq 104+$ m for testing individual predictors (Tabachnick & Fidell, 2007). Thus, the sample size in this study (N = 380) was sufficient for analysis.

The goal of multiple regression is to understand each predictors (independent variable) relationships to prenatal depressive symptoms. The suppression effects among predictors were examined to limit irrelevant elements between predictors and criterion (Beckstead, 2012).

Regression analysis is a statistical technique used for studying linear relationship, predicting the value of one dependent variable from the value of one independent variable. Assumptions for regression analysis include: the variables have normal distribution (normality), the predictors are linearly independent (linearity), the errors are uncorrelated (homoscedasticity), and the error is independent (Tabachnick & Fidell, 2007).

Steps involved in regression analysis include: (1) state the research hypothesis and null hypothesis, (2) assess normality on each variable separately, (3) assess the linear relationship of the independent variable and the dependent variable (correlation coefficient), (4) estimates the regression equation from data, (5) estimates and examine appropriate measures of association

and test of statistical significance for each coefficient and for the equation, (6) accept or reject the null hypothesis, (7) accept or reject research hypothesis.

Analyses to address the hypothesis. Multiple regressions were conducted to evaluate the influence of variables (perceived stress, herpes viral titers) on the dependent variable (depressive symptoms) and metabolites of the IDO and GTP-CH1 pathways while controlling for covariate variables. To address the three hypotheses, following data analysis methods were used:

Hypothesis 1. There is a significant positive relationship between stress and depressive symptoms in pregnant women. The tentative hypothesis is that higher levels of stress increased severity of depressive symptom in pregnant women. Regression analysis was used to explore the relationship between stress and dysphoric moods such as depressive or fatigue symptoms during pregnancy. The independent variable was perceived stress and the dependent variable was depressive symptoms. The regression is given by: $(Y'_1) = a + b_1 (x_1)$. Y'_1 (POMS-D) can be expressed in terms of a constant (a) and a slope (b_1) times the ($x_{1,\ stress}$) variable. The goal of this regression was to assess the value and significance of coefficient values, controlling for demographics, BMI, and number of children.

Hypothesis 2. There are significant positive relationships among herpes viral titers, stress and depressive symptoms in pregnant women. The tentative hypothesis is that the severity of depressive symptoms increases as the level of stress increases, and this positive relationship is partially mediated by herpes viral titers in pregnant women.

The independent variable was perceived stress. The dependent variable was depressive symptoms (POMS-D), and herpes viral titers (HSV-1, HSV-2, HHV-6, CMV, and EBV) are the mediator /intervening variable (Table 2). Multiple regression analyses were used to explore the relationship between stress, depression and herpes viral titers:

Depression ($Y_{2.1}$) = a +b_1 (stress) + b_2 (herpes viral titers)

Mediation analysis. The three regression paths for testing mediational effect were (Baron & Kenny, 1986): (1) regressing the dependent variable (depressive symptoms) on the independent variable (perceived stress), (2) regressing the mediator (herpes viral titers) on the independent variable (perceived stress), and (3) regressing the dependent variable (depressive symptoms) on both the mediator (herpes viral titers) and the independent variable (perceived stress).

Table 2
Data Analysis

Hypothesis	Regression
1.	Stress→ Depression
2.	Stress→Herpes viral titers →Depression
3.	Stress→Herpes viral titers→ KYN /TRP ratio →Depression
	Stress→ Neopterin→Phe /Tyr ratio→Depression

Note. KYN /TRP: kynurenine /tryptophan, Phe /Tyr: Phenylalanine /Tyrosine

Hypothesis 3. The kynurenine /tryptophan ratios (KYN /TRP) and phenylalanine /tyrosine ratios (Phe /Tyr), and neopterin levels are increased positively related to herpes viral titers in depressed pregnant women. Multiple regression analysis was used to explore the relationship between stress, neuroimmune biogenic amines, herpes viral titers, and depression (Table 2).

For stress, depression, herpes viral titer and KYN /TRP ratio:

Depression ($Y_{3.1}$) = a +b_1 (stress) + b_2 (herpes viral titers) +b_3 (KYN /TRP)

For stress, depression, herpes viral titer and level of neopterin:

Depression ($Y_{3.6}$) = a +b_1 (stress) + b_2 (herpes viral titers) +b_3 (KYN /TRP) +b_4 (neopterin)

For stress, depression, herpes viral titer, and Phe /Tyr ratio:

Depression ($Y_{3.11}$) = a +b_1 (stress) + b_2 (herpes viral titers) +b_3 (KYN /TRP) +b_4 (neopterin) + b_5 (Phe /Tyr)

Figure 3 summary hypothesized model of prenatal depression in the current study.

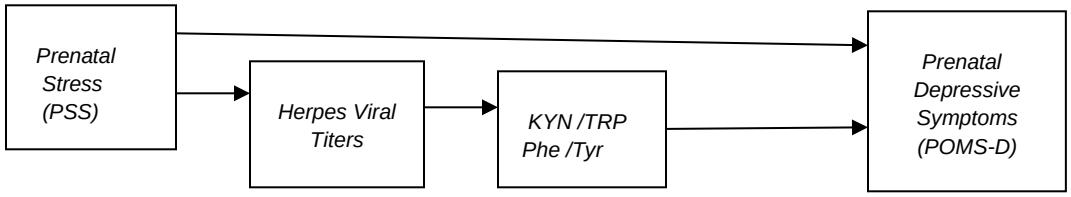


Figure 3. Hypothesized relationships in prenatal stress, herpes viral titers leading to biogenic amines alteration in prenatal depressive symptoms.

Chapter Summary

Influence of Lactation on Postpartum Stress and Immunity study was the foundation for this study on relationships of prenatal stress, depression and herpes viral titers. It provided baseline data from women collected at 16 to 25 gestational weeks that was used for secondary data analysis. This original data set includes PSS, POMS, neuroimmune biogenic amines; HSV-1, HSV-2, HHV-6, CMV herpes viral titers. Additional EBV viral titers were measured in stored plasma samples. The aim of this study was to examine relationships among stress, herpes viral reactivation, the IDO activation pathway, and depression.

CHAPTER FOUR

RESULTS

Preliminary Analyses

Screening and cleaning data. Total samples of 640 pregnant women were in the prenatal R01 electronic SPSS data file. To avoid the risk of affecting statistical power and increasing chance of making Type I or Type II errors, incomplete data sets were not included in the final analysis. These included variables such as education, income, marital status, work status, smoking, and biogenic amines and herpes viral titers which were available on 431 women. After filtering out missing data, a full set total of 380 samples were included in this data analysis. The variables in this study are: demographic data (age, race, gravidity, number of children, BMI); self- report questionnaires (Perceived Stress Scale, Profile of Mood States); herpes viral antibody IgG titers (HSV-1, HSV-2, HHV-6, CMV); biogenic amines (tryptophan, kynurenine, kynurenine /tryptophan ratio (KYN /TRP), phenylalanine, tyrosine, phenylalanine /tyrosine ratio (Phe /Tyr), nitrite, and neopterin), and potential covariate variables (thyroid peroxidase antibody, *Toxoplasma gondii* IgG titer). All EBV viral capsid antigen (VCA) antibody IgG titers were performed by the PhD candidate and added in this study. A total sample $N = 219$ EBV VCA IgG titer was added to the data set.

The data screening processes were conducted in two steps: (1) checking each variable for scores that are out of range by checking minimum and maximum values, mean, standard deviation, and frequencies, (2) finding and correcting the error in the data file.

Description of the sample. Variable descriptive statistics including mean, standard deviation, range of scores, skewness, kurtosis and normality were assessed. The participants were in the early second trimester, averaging 19 weeks of pregnancy. As shown in Table 3, participants' ages ranged from 17 to 45 years. The most common race reported was Caucasian. Approximately 23.2 % of participants identified themselves as African American /Black and 22.9 % as Hispanic. The number of children reported by participants ranged from 0 to 6. Nearly 45.5 % of participants have no child at home, 31.3 % have one child, and 15.5 % have two children at home.

Participants pre-pregnancy body mass index (Pre-BMI) was calculated using self-reported pre-pregnancy weight and height and range from 17.2 to 58.3 (*Mean* = 27.01, *SD* = 6.80). Upon enrollment in this study, participants BMI ranged from 18.7 to 60 (*Mean* = 28.85, *SD* = 6.48). Pre-pregnancy BMI was highly correlated with BMI upon enrollment (r = .933, p= .000). The accuracy of BMI was affected by pregnancy related weight gain, so pre-pregnancy BMI was used in this statistical analysis. Total of 375 BMI data were available in this study.

The Perceived Stress Scale (PSS) data from participants responses were on 1 to 5 Likert-type scale (1 = never, 2 = almost never, 3 = sometimes, 4 = fairly often, 5 = very often). The scores on the seven positive questionnaires were reversed and all the questionnaires were summed to calculate a total score in SPSS data file. The PSS score in this study had a range of 2 to 45 (*Mean* = 22.04, *SD* = 7.13). Cronbach's alpha (α) coefficient in this study was .85.

Participants' responses from the 65 -items Profile of Mood State (POMS) scale were collected and then each subscale was calculated. The POMS depression-dejection subscale score (POMS-D) is a 15- item scale which ranged from 0 to 60. The POMS-D in this study had a range from 0 to 49 (*Mean* = 5.72, *SD* = 8.51), Cronbach's alpha coefficient was .91. African American

women had higher depression scores than Caucasian or Hispanic women (Table 3). Twenty-five participants (6.6 %) POMS-D scores were over 20 which indicated clinical depression and they were referred to their provider for further depression evaluation and treatment. POMS fatigue-inertia (POMS-F) is a 7-item scale which ranged from 0 to 28. The POMS-F in this study had a range from 0 to 25 (*Mean* = 8.04, *SD* = 5.38), Cronbach's alpha coefficient was .87.

Table 3
Sample Demographic Characteristics and Psychosocial Measures (N=380)

Variable	*N*	%	Min-Maximum	*M*	*SD*	POMS-D Score *M*	POMS-D Score *SD*
Age			17-45	28.22	5.99		
Race							
Caucasian	179	47.1				4.05	6.44
African America	88	23.2				7.85	11.62
Hispanic	87	22.9				6.90	7.89
Asian	11	2.9				5.09	7.40
Native America	2	0.5				0.00	0.00
Other	13	3.4				8.69	9.89
Number of Children			0 - 6	0.92	1.14		
0	173	45.5					
1	119	31.3					
2	59	15.5					
3	12	3.2					
4	11	2.9					
5	3	0.8					
6	3	0.8					
BMI			17.20-58.30	26.98	6.85		
Perceive Stress Scale (PSS)			2-45	22.04	7.13		
POMS-Depression			0-49			5.72	8.51
POMS-Fatigue			0-25			8.04	5.38

Note. BMI: Body mass index, POMS: Profile of Mood States, *M*: Mean, *SD*: Standard deviation
Cronbach's α = PSS (.85), POMS-D (.91), POMS-F (.87)

Biological variables. Raw data of biological variables including herpes viral titers and biogenic amines are summarized in Table 4. The serological herpes viral titers (HSV-1, HSV-2, HHV-6, and CMV) cut-off point was taken from the Stanley Virology Laboratory of Johns Hopkins University headed by Dr. Robert Yolken (Dickerson et al., 2003; Groer et al., 2011) for

quantitative analysis. The cut-off point for seropositivity to HSV-1, HSV-2, and CMV was 1 and for HHV-6 was 6. The EBV VCA IgG enzyme-linked immunosorbent assay (ELISA) was performed at College of Nursing, University of South Florida. EBV IgG was measured by optical density ratio units and calculated according to manufacturer (ALPCO). The cut-off point for EBV was 10 and intra-assay coefficients of variation were less than 10 % (Table 5). The above threshold antibody titers (seropositive) to different herpes viruses were analyzed as a continuous variable when relationships among stress, depression, and biogenic amine pathways were examined.

Table 4
Biological Variables (N =380)

Variable	Min-Maximum	*M*	*SD*
Herpes Viral Titers			
Herpes Simplex Virus type 1 (HSV-1)	0.07- 7.76	2.97	2.62
Herpes Simplex Virus type 2 (HSV-2)	0.05- 13.60	1.27	2.53
Human Herpes Virus-6 (HHV-6)	0.30- 29.53	11.09	6.17
Cytomegalovirus (CMV)	0.13- 10.52	2.73	2.54
Epstein-Barr Virus (EBV♯)	3.62- 39.25	23.24	8.75
Biogenic Amine			
Neopterin (Neo)	2.93- 18.69	5.83	2.05
Nitrite (Nitr)	2.00- 87.00	19.13	13.85
Tryptophan (TRP)	8.00-122.00	62.28	14.72
Kynurenine (KYN)	0.00- 5.00	1.90	0.74
Kynurenine/ Tryptophan ratio (KYN /TRP)	8.00- 72.00	31.10	11.50
Phenylalanine (Phe)	18.00-132.00	52.34	15.08
Tyrosine (Tyr)	5.00-130.00	43.13	16.98
Phenylalanine /Tyrosine ratio (Phe /Tyr)	0.00- 9.00	1.09	0.49
Other Potential Covariate			
Toxoplasma gondii (toxo)	0.90- 4.17	0.45	0.88
Thyroid peroxidase antibody (TPO)	0.00- 12.27	0.11	0.68

Note. EBV♯ *N* = 219, *M*: Mean, *SD*: Standard deviation

Table 5
Variables Threshold Cut-off point (N =380)

Variable	Cut-off Point	Below Cut-off Point		Over Cut-off Point	
		N	%	*N*	%
Profile of Mood States- Depression	20	355	93.4	25	6.6
Herpes simplex virus type 1	1	163	42.2	217	57.8
Herpes simplex virus type 2	1	297	78.0	83	22.0
Human herpes virus-6	6	90	23.8	290	76.2
Cytomegalovirus	1	158	39.9	222	60.1
Epstein-Barr virus (EBV♮)	10	21	9.3	198	90.7

Note. EBV♮ *N* = 219

Bivariate data analysis (Pearson correlation) was conducted to assess and explore the variables relationships among dysphoric moods (POMS-depression, fatigue, anxiety, anger, vigor, and confusion) and the significance of coefficient values (Table 6). When EBV IgG titers were included in the Pearson correlation, a total only 219 data were available to analyze. TPO (r = .055, p = .285, two-tailed), *T. gondii* titers (r = .037, p = .477), BMI (r = .081, p = .117) and EBV IgG titers (r = -.078, p = .251) were not significantly correlated to POMS-D or other key variables. Due to the sample size and to avoid the risk of affecting statistical power, BMI and EBV antibody titers were not included in the final hypotheses analysis.

The final 380 women with the 17 data variables were entered in the Pearson correlation analysis. Table 6 provides correlations for all linear variables. The perceived stress scores (PSS) were highly positively correlated to POMS-D (r =.601, p =.000) and POMS-F (r =.43, p =.000) and negatively correlated to age (r =-.125, p =.015). POMS-D scores were positively correlated to POMS-F (r =.55, p =.000) and number of children (r =.173, p = .001), HSV-2 (r = .215, p =.000), and negatively correlated with HHV-6 (r = -.130, p = .011) and phenylalanine (r = -.152, p =.003) (Table 6).

Table 6

Correlation of Key Variables in the Current Study (N=380)

Variable	POMS-D	POMS-F	PSS	Age	#childr	HSV-1	HSV-2	HHV-6	CMV	Neo	Nitr	TRP	Kyn	Kyn/Trp	Phe	Tyr	Phe/Tyr
POMS-D	1																
POMS-F	.55‡	1															
PSS	.60‡	.43‡	1														
Age	.01	.01	-.13*	1													
#childr	.17‡	.05	.14‡	.25‡	1												
HSV-1	.03	-.16‡	.05	.01	.27‡	1											
HSV-2	.22‡	.04	.11*	.08	.18‡	.14‡	1										
HHV-6	-.13*	.02	-.11*	-.01	-.20‡	-.30‡	-.15‡	1									
CMV	.03	-.06	.01	.03	.16‡	.23‡	.22‡	-.09	1								
Neo	-.02	.09	.00	.06	.08	.01	-.06	.08	.13‡	1							
Nitr	.05	.01	.05	.02	.09	.04	-.02	-.09	.01	-.04	1						
TRP	-.07	-.04	-.07	.02	-.15‡	-.15‡	-.09	.16‡	-.04	.06	.06	1					
KYN	-.00	.08	.03	.03	-.01	-.04	-.05	.13*	-.03	-.36‡	-.04	.38‡	1				
Kyn/Trp	.04	.08	.08	-.01	.10*	.07	-.01	.01	-.01	.33‡	-.07	-.24‡	.77‡	1			
Phe	-.15‡	-.03	-.13*	.05	-.09	-.14‡	-.10*	.16‡	-.07	.16‡	.15‡	.63‡	.23‡	-.16‡	1		
Tyr	-.06	-.01	-.03	.03	-.07	-.08	-.06	.05	.04	-.07	.06	.54‡	.07	-.26‡	.70‡	1	
Phe/Tyr	-.02	-.06	.06	.12*	.01	-.08	-.05	-.12*	-.11*	.17‡	-.01	.01	.09	.09	.12*	.33*	1

Note. POMS-D: Profile of Mood States-Depression, POMS-F: Profile of Mood States-Fatigue, PSS: perceived stress scale, #childr: Number of children, HSV-1: Herpes simplex virus type 1, HSV-2: Herpes simplex virus type 2, HHV-6: Human herpes virus-6, CMV: cytomegalovirus, Neo: Neopterin, Nitr: Nitrite, TRP: Tryptophan, KYN: Kynurenine, Kyn /Trp: Kynurenine/ Tryptophan, Phe: Phenylalanine, Tyr: Tyrosine, Phe /Tyr: Phenylalanine /Tyrosine

N=380, Listwise. *$P < .05$ ‡$P < .01$, two-tailed test.

The variables that were significantly related to POMS-depressive symptoms or POMS-fatigue subscale scores were entered into multiple regression analysis to examine the relationships between stress, herpes viral titers, and depressive symptoms or fatigue symptoms.

Depressive Symptoms and Biomarkers

Initial analyses of independent t-tests were conducted to assess two groups of women, POMS-D score > 20 and POMS-D score ≤ 20 (Table 7). The PSS scores, biomarkers, herpes viral titers and biogenic amines levels were compared. The clinically depressed women (POMS-D > 20) had a significantly higher PSS scores and HSV-2 titers than the women with POMS-D score ≤ 20. They also had a lower HHV-6 titers, tryptophan and phenylalanine levels than the women with POMS-D score ≤ 20. Additionally, clinically depressed women had higher nitrite, KYN /TRP ratios, HSV-1, CMV, EBV antibody titers and lower neopterin, kynurenine, tyrosine, and Phe /Tyr ratios but they none were statistically significant ($p > .05$). Among twenty-five clinically depressed (POMS-D > 20) women, ten were African American and seven were Hispanic.

Data Analysis to Address the Hypothesis

Hypothesis 1

There is a significant positive relationship between stress and depressive symptoms in pregnant women.

Log-10 transformed POMS-D score were used in the hypothesis analysis to correct skewness and better meet the assumption of the normality. The regression equation is given by: $(Y'_1) = a + b_1 (x_1)$. Y'_1 (POMS-D) can be expressed in terms of a constant /intercept (a) and a slope (b_1) times the (x_1) variable. When the independent variable perceived stress (PSS) alone is entered into the regression analysis, the regression equation is given by:

($Y'_{1)}$ = -.249 +.037 (PSS). The perceived stress (β = .575, p = .000, R^2 = .331, adjusted R^2 = .329) was a strong influence on the POMS-D scores (Figure 4).

Table 7
Summary T-Tests of Variables in Women with POMS-D Score > 20 and POMS-D Score ≤ 20 Group

Variable	Score	*M*	*SD*	*t*	Mean Difference	95% *CI*	η^2
PSS Score	POMS-D > 20	34.80	5.94	10.50**	13.66	11.10 to 16.22	.224
	≤ 20	21.14	6.31				
HSV-1	POMS-D > 20	3.57	2.72	1.19			
	≤ 20	2.92	2.62				
HSV-2	POMS-D > 20	3.18	4.26	2.36*	2.04	.27 to 3.81	.015
	≤ 20	1.14	2.31				
HHV-6	POMS-D > 20	7.62	4.07	-4.23**	-3.71	-5.50 to -1.92	.045
	≤ 20	11.33	6.22				
CMV	POMS-D > 20	2.70	2.61	-.09			
	≤ 20	2.75	2.53				
EBV♮	POMS-D > 20	21.06	9.69	-.96			
	≤ 20	23.39	8.69				
Nitrite	POMS-D > 20	21.52	14.63	.89			
	≤ 20	18.96	13.80				
Neopterin	POMS-D > 20	5.41	1.37	-1.05			
	≤ 20	5.85	2.11				
Tryptophan	POMS-D >20	54.70	10.81	-2.69**	-8.11	-14.05 to -2.17	.018
	≤ 20	62.81	14.82				
Kynurenine	POMS-D > 20	1.82	.68	-.56			
	≤ 20	1.90	.75				
KYN /TRP	POMS-D > 20	33.35	11.53	1.03			
	≤ 20	30.94	11.50				
Phenylalanine	POMS-D > 20	42.93	13.51	-3.27***	-10.01	-16.06 to -4.01	.027
	≤ 20	53.00	14.98				
Tyrosine	POMS-D > 20	45.02	14.29	-.17			
	≤ 20	51.06	17.05				
Phe /Tyr	POMS-D > 20	.98	.21	-1.18			
	≤ 20	1.09	.50				

Note. M: Mean, *SD*: Standard deviation, PSS: Perceived Stress Scale, POMS-D: Profile of Mood States-Depression, HSV-1: Herpes simplex virus type 1, HSV-2: Herpes simplex virus type 2, HHV-6: Human herpes virus-6, CMV: Cytomegalovirus, EBV: Epstein-Barr virus, KYN/TRP: Kynurenine /Tryptophan, Phe/Tyr: Phenylalanine /Tyrosine *df* = 378, *P <.05, **P <.01, *** P < .001, two-tailed. EBV♮ *df* =217.

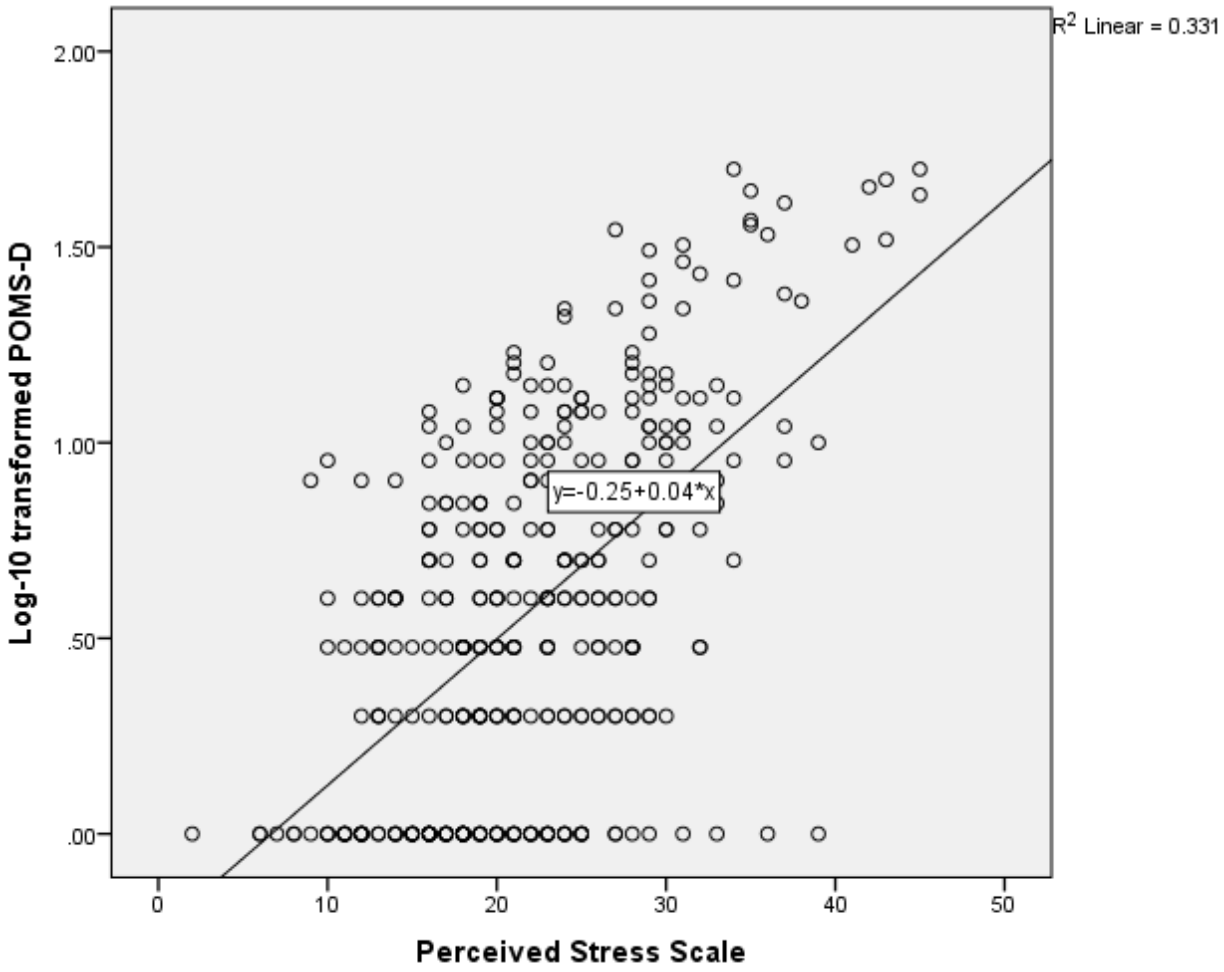


Figure 4. Scatter plot of log-10 transformed Prenatal Profile of Mood States-Depression (POMS-D) scores (Y-axis) and perceived stress scores (X-axis) in a linear regression relationship

Perceived stress and demographic variables. The perceived stress was negatively correlated to age (r = -.125, p = .015) (Table 6). The independent demographic variables (age, African American, number of children) and perceived stress were entered to assess interference with dependent variable depressive symptoms. To examine the possible suppression effect among demographic variables and perceived stress, they were entered separately in the regression model. R squared (R^2), standardized regression weight, zero-order and the partial correlation coefficient sign and size were compared. The results showed that being African

American (AA), number of children and age had some degree of correlation. They also correlated with perceived stress. The age variable was a major suppressor; as it suppressed other independent variables. Therefore, the age was removed from regression analysis to reduce the potential suppression effect. The perceived stress was positively associated with number of children (β = .147, p = .006) and the African American race (β = .123, p = .022).

Depressive symptoms and perceived stress. Hierarchical multiple regression was used to assess the ability of prenatal perceived stress in predicting levels of depressive symptoms after controlling for race (African American) and number of children. Independent variables African American race and number of children were entered at Step 1, explaining 1.3 % of the variance in depressive symptoms. After entry of the perceived stress in Step 2 the total variance explained by the model as a whole was 33.1 %. The perceived stress explained the additional 31.8 % of the variance. Table 8 summarizes the multiple regressions analyses. The results suggest perceived stress (β = .577, p = .000) strongly predicted depressive symptoms.

Table 8
Relationship between Prenatal Depressive Symptoms and Perceived Stress (N=380)

	Step 1			Step 2		
Variable	*B*	*SE B*	β	*B*	*SE B*	β
Intercept	.529	.032		-.250	.064	
AA	.092	.057	.083	-.012	.048	-.011
#Children	.026	.021	.064	.001	.017	.002
PSS				.037	.003	.577***
	R^2 = .013			R^2 = .331		
	Adjusted R^2 = .008			Adjusted R^2 = .326		
	F(2, 377) = 2.501			F(3, 376) = 62.054***		
	R^2 change = .318					
	F change (1, 376) = 178.800***					

Note. AA: African American, #Children: Number of children, PSS: Perceived Stress Scale
***P<.001

Summary of hypothesis 1. As expected, there was a significantly positive relationship between perceived stress and depressive symptoms in pregnant women. The key predictor, perceived stress, accounted for 31.8% of the variance in prenatal depression model. Women in the African American group showed higher stress scores. Also, the more children pregnant women had, the more stress they had.

Hypothesis 2

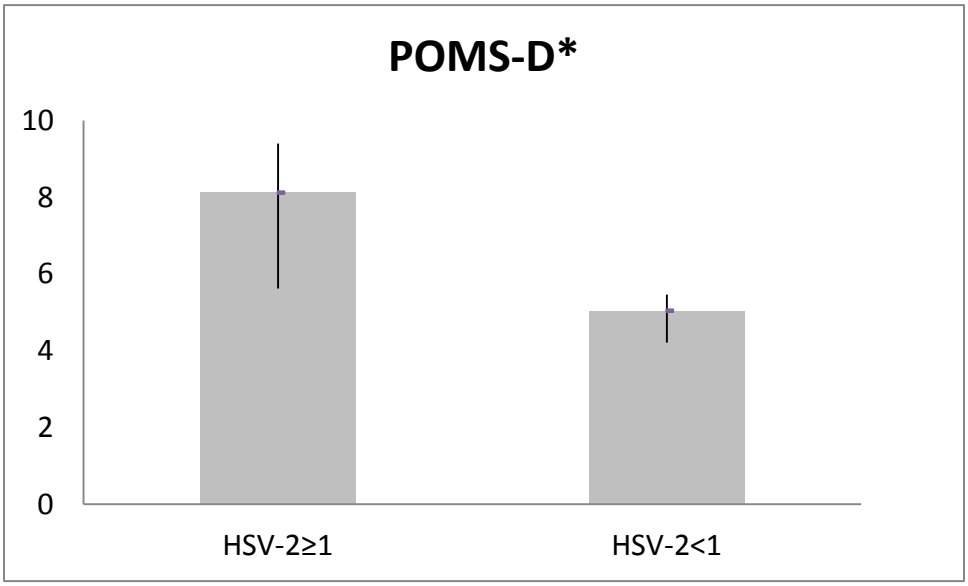
There are significant positive relationships among herpes viral titers, stress and depressive symptoms in pregnant women.

During this exploratory analysis, independent sample t-tests, Pearson correlation and multiple regression analysis were used to understand each herpes viral seroprevalence status in association with prenatal perceived stress and depressive symptoms.

HSV-1 seropositive titers, perceived stress and depressive symptoms. Independent variables race (African American), number of children and perceived stress were entered at Step 1 (R^2 = .331, F (3, 376) = 62.054, p = .000). After entry of the HSV-1 seropositive titers in Step 2, (R^2 = .332, F (4, 375) = 46.495, p = .000), R^2change = .000, F change (1, 375) = .209, p = .648), the HSV-1 seropositive titers ((β = .020, p = .648) were not significantly associated with depressive symptoms. However, perceived stress (β = .577, p = .000) remained significantly associated with depressive symptoms.

Separate multiple regression analysis was used to explore relationship between perceived stress and HSV-1 seropositive titers. The dependent variable HSV-1 seropositive titers and independent variables PSS, race (American African), number of children were entered in the regression analysis (R^2 = .074, F (3, 376) = 9.954, p = .000), the relationship between HSV-1 seropositive titers and PSS (β = -.011, p = .823) were not significant.

HSV-2 seropositive titers, perceived stress and depressive symptoms. From independent sample t-test analysis, the women with HSV-2 titers ≥ 1 (seropositive) had a significantly higher POMS-D score than the women with HSV-2 titers < 1 (seronegative) (Figure 5 & Table 9). From Pearson correlation analysis, HSV-2 titers were correlated with POMS-D score (r = .215, p = .000, two-tailed), respectively (Table 6).



*P < .05

Figure 5. Significantly higher Profile of Mood States - Depression (POMS-D) scores in women with herpes simplex virus type 2 (HSV-2) seropositive titers

Table 9
Difference Scores in Women with Herpes Viral Titers above and below Threshold

Variables	Viral Serology	*M*	*SD*	*t*	Mean Difference	95% *CI*	η^2
POMS-Depression	HSV-2 ≥ 1	8.12	11.63	2.29*	3.08	.41 to 5.75	.014
	HSV-2 < 1	5.04	7.29				
Perceived stress scale (PSS)	HHV-6 ≥6	21.63	7.11	-2.02*	-1.74	-.3.42 to -.049	.011
	HHV-6 <6	23.37	7.09				
POMS-Fatigue	CMV ≥ 1	7.32	5.21	-3.15**	-1.74	-2.83 to-.66	.015
	CMV < 1	9.06	5.47				

Note. HSV-2: Herpes simplex virus type 2, HHV-6: Human herpes virus-6, CMV: Cytomegalovirus, POMS: Profile of Mood States, df = 378, *P <.05, ** P <.01, *** P <.001, two-tailed.

The dependent variable log -10 transformed POMS-D scores and independent variable HSV-2 seropositive titers (HSV-2≥ 1) only were entered into regression analysis (*N*=380). The results showed that HSV-2 seropositive titers (β = .102, p = .046), R^2 =.010, F (1, 378) = 3.995, p = .046) significantly predicted depressive symptoms. HSV-2 seropositive titers remained significant to predict depressive symptoms (β = .085, p = .050), R^2 =.338, F (3, 376) = 63.979, p = .000) after independent variables race (African American) and PSS were added.

Hierarchical multiple regression was used to understand the relationship between HSV-2 seropositive titers and depressive symptoms. The full sample included independent variables race (African American), number of children and perceived stress which were entered at Step 1, explaining 33.1 % of the variance in depressive symptoms (R^2 =.331, F (3, 376) = 62.054, p = .000). After entry of the HSV-2 seropositive titers at Step 2 total variances explained by the model as a whole was 33.8 % (R^2 = .338, F (4, 375) = 47.868, p = .000). The HSV-2 seropositive titers (β = .086, p = .050) were significantly related to depressive symptoms (R^2 change= .007, F change (1, 375) = 3.882, p = .050) (Table 10).

Table 10
Predictors of Prenatal Depressive Symptoms (N=380)

	Step 1			Step 2		
Variable	*B*	*SE B*	β	*B*	*SE B*	β
Intercept	-.250	.064		-.264	.064	
AA	-.012	.048	-.011	-.034	.049	-.031
#children	.001	.017	.002	-.003	.017	-.007
PSS	.037	.003	.577***	.038	.003	.578***
HSV-2 ≥1				.096	.049	.086*
	R^2 = .331			R^2 = .338		
	Adjusted R^2 = .326			Adjusted R^2 = .331		
	F(3, 376) =62.054***			F(4, 375) = 47.868***		
	F change (1, 375) = 3.882*					

Note. AA: African American, #children: Number of children, PSS: Perceived Stress Scale, HSV-2: Herpes simplex virus type-2, *P <.05, ***P <.001

To explore relationship between perceived stress and HSV-2 seropositive titers in full sample (N = 380), the dependent variable HSV-2 seropositive titers and independent PSS, race (African American) and number of children were entered (R^2= .074, F (3, 376) = 10.280, p = .000), the relationship between HSV-2 seropositive titers and PSS (β = -.015, p = .768) were not significant.

Women with higher HSV-2 seropositive titers had more depressive symptoms. Table 11 provides Pearson correlation analysis of key variables in women with HSV-2 seropositive titers (N=83). The HSV-2 seropositive titers were entered as a continuous variable in a separate regression analysis to examine whether women with higher HSV-2 seropositive titers had more depressive symptoms. The results revealed that HSV-2 seropositive titers (β = .227, p = .039), R^2= .051, F (1, 81) = 4.392, p = .039) strongly predicted depressive symptoms.

The hierarchical multiple regression was used and independent variables race (African American), number of children and HSV-2 seropositive titers were entered one at a time. The size and sign of the independent variables zero-order and partial order were examined to identify potential suppression effects. The number of children variable was a negative suppressor. The number of children has positive correlation with other independent and dependent variables, however when entered into multiple regression analysis, the number of children had a negative β weight. HSV-2 seropositive titers (β = .249, p = .028) were significantly correlated with depressive symptoms after controlling for race (African American) and number of children (Figure 6 & Table 12).

Table 11

Correlation of key Variables in Women with HSV-2 Seropositive Titers (N=83)

Variable	POMS-D	PSS	Age	#childr	HSV-1	HSV-2	HHV-6	CMV	Neo	Nitr	TRP	Kyn	Kyn/Trp	Phe	Tyr	Phe/Tyr
POMS-D	1															
PSS	.70‡	1														
Age	-.05	-.20	1													
#childr	.09	.15	.15	1												
HSV-1	.09	.12	-.06	.31‡	1											
HSV-2	.23*	.24*	.01	.15	.28‡	1										
HHV-6	- .05	-.09	-.07	-.16	-.32‡	-.24*	1									
CMV	.06	.08	.11	.10	.09	.28*	-.17	1								
Neo	-.02	-.07	.04	-.06	-.03	.09	.05	.21	1							
Nitr	.01	-.06	.19	.06	.00	-.13	.23*	.10	-.05	1						
TRP	-.14	-.04	-.00	-.17	-.32‡	-.09	.15	.15	.07	-.12	1					
KYN	-.04	.09	-.17	-.15	-.19	-.13	.08	.11	.21	-.08	.43‡	1				
Kyn/Trp	.14	.14	-.25*	-.02	.05	-.04	-.00	.00	.20	-.06	-.13	.81‡	1			
Phe	-.28*	-.26*	.05	-.13	-.37‡	-.13	.29‡	-.04	.12	.02	.67‡	.25*	-.13	1		
Tyr	-.23*	-.18	.03	-.15	-.31‡	-.13	.04	.11	.05	-.15	.74‡	.23*	-.20	.73‡	1	
Phe/Tyr	-.12	-.13	.12*	.01	-.12	.02	.37‡	-.17	.12	.26*	-.05	.02	.09	.41*	-.30‡	1

Note. POMS-D: Profile of Mood States-Depression, PSS: perceived stress scale, #childr: Number of children, HSV-1: Herpes simplex virus type 1, HSV-2: Herpes simplex virus type 2, HHV-6: Human herpes virus-6, CMV: cytomegalovirus, Neo: Neopterin, Nitr: Nitrite, TRP: Tryptophan, KYN: Kynurenine, Kyn/Trp: Kynurenine/ Tryptophan, Phe: Phenylalanine, Tyr: Tyrosine, Phe/Tyr: Phenylalanine /Tyrosine

N=83, Listwise. **P* < .05 ‡*P* < .01, two-tailed test.

Table 12
Predictors of Prenatal Depressive Symptoms in HSV-2 Seropositive Women (N=83)

Variable	*B*	*SE B*	β
Intercept	.528	.116	
AA	.034	.122	.032
# Children	-.078	.042	-.211
HSV-2≥1 Titers	.042	.019	.249*
	R^2 = .092		
	Adjusted R^2 = .057		
	F (3, 79) = 2.662*		

Note. AA: African American, #children: Number of children, PSS: Perceived Stress Scale, HSV-2: Herpes simplex virus type-2, **P* <.05

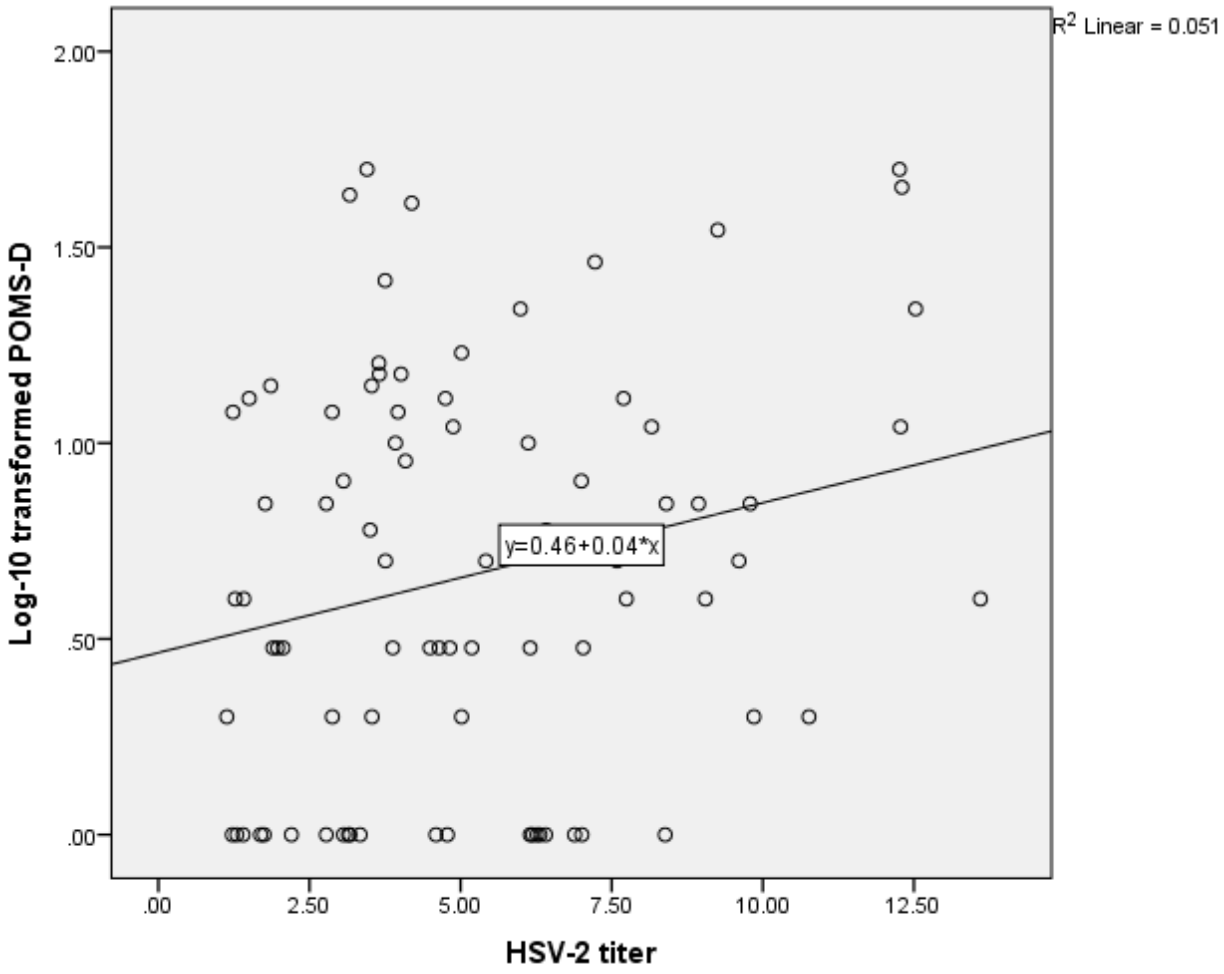


Figure 6. Scatter plot of herpes simplex virus type 2 titers (HSV-2) (X-axis) and log-10 transformed Profile of Mood States- Depression scores (POMS-D) (Y-axis) in seropositive women

Women with higher perceived stress also had higher HSV-2 seropositive titers.

Relationships between perceived stress and herpes seropositive titers were examined in this separate analysis. The dependent variable HSV-2 seropositive titers (N = 83) and independent variable PSS were entered in the regression model to explore whether the women with higher perceived stress had higher HSV-2 seropositive titers. The perceived stress (β = .235, p = .032) was significantly positively associated to HSV-2 seropositive titers (R^2= .055, adjusted R^2= .044, F (1, 81) = 4.756, p = .032). The higher perceived stress the pregnant women had, the higher HSV-2 titers they had (Figure 7).

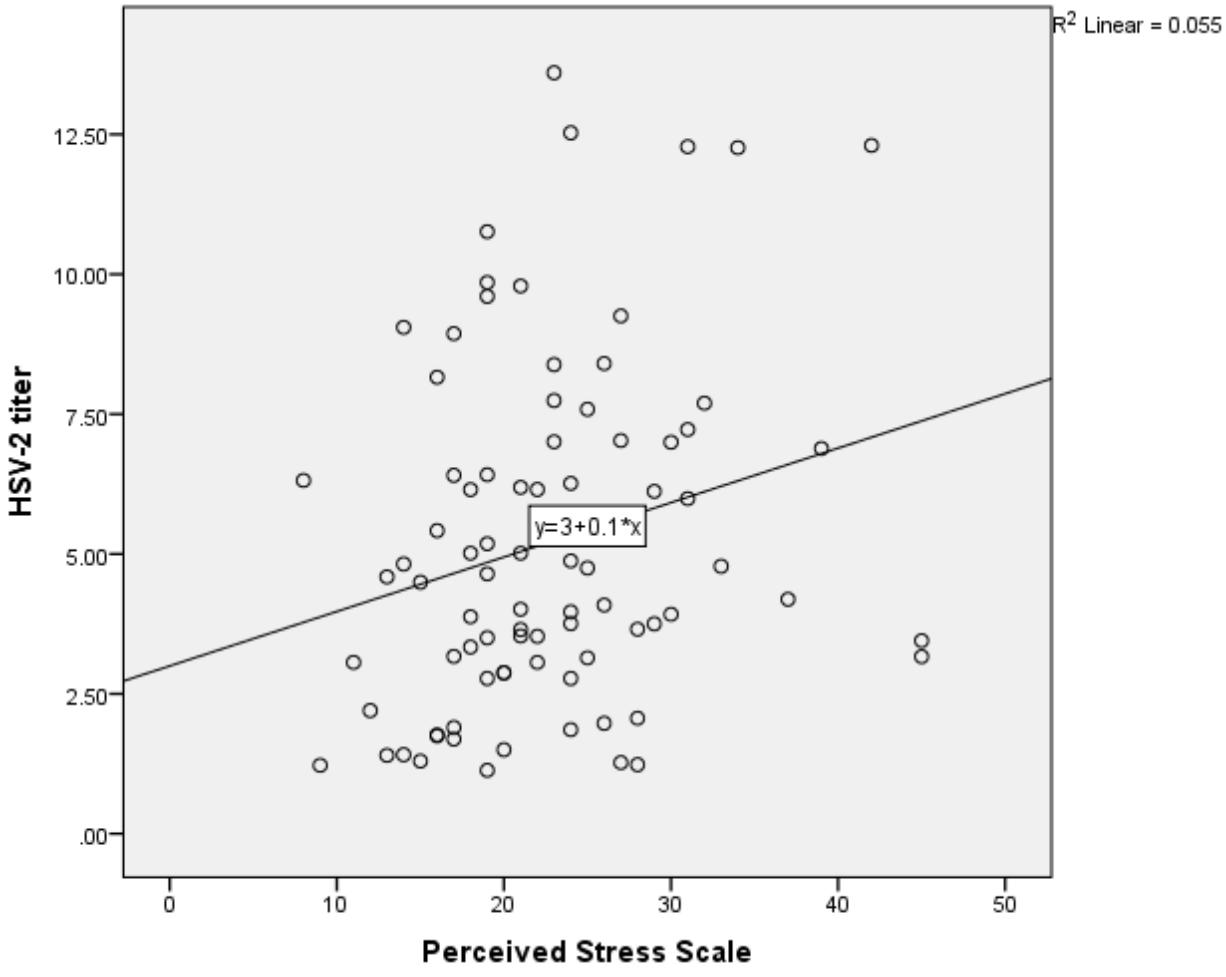


Figure 7. Scatter plot of herpes simplex virus type 2 (HSV-2) titers (Y-axis) and Perceived Stress Scale scores (X-axis) in seropositive women

The herpes viral titer (HSV-2) was a mediator between perceived stress and depressive symptoms. The mediation analysis of three regression equations provided evidence that the herpes viral titer (HSV-2) was a mediator, partially mediating the effect of perceived stress on depressive symptoms. Based on the results of hypothesis 1 and hypothesis 2, these regression equations can be summarized as follow: (1) perceived stress strongly predicted depressive symptoms (β = .577, p = .000) (Figure 4 & Table 8), (2) the higher perceived stress significantly predicted higher HSV-2 seropositive titers (β = .235, p = .032) (Figure 7), (3) the perceived stress (β = .578, p = .000) and HSV-2 seropositive titers (β = .086, p = .05) significantly predicted depressive symptoms (Table 10).

Regression analysis showed that both perceived stress and HSV-2 seropositive titers strongly predicted depressive symptoms (Table 10). This indicated that there were multiple mediating factors in this model. The severity of depressive symptoms increases as the level of stress increases, and this positive relationship was partially mediated by herpes viral titers in pregnant women; herpes viral titer (HSV-2) was the mediator /intervening variable.

Characteristics of women with HSV-2 seropositive titers. Among the HSV-2 seropositive women (N = 83), the age ranged from 19-45 years old. African American (43.3 %) women had higher seroprevalence for the HSV-2 infection. Ten out of eighty-three HSV-2 seropositive women had a POMS-D score over 20 and were referred to their healthcare providers. Among HSV-2 seropositive women, 77.1 % also had the CMV infection, 71.1 % had the HHV-6 infection, 67.5 % had the HSV-1 infection and 67.5 % had EBV infection (Table 13).

Table 13
Demographic Characteristics and Herpes Viral Titers in Women with HSV-2 Seropositivity (N=83)

Variable			HSV-2 Titer		POMS-D Score	
	N	%	*M*	*SD*	*M*	*SD*
Race						
Caucasian	19	22.9	3.65	2.78	4.89	5.58
African America	36	43.4	6.09	3.37	10.19	15.20
Hispanic	22	26.5	5.66	2.42	8.50	9.54
Asian	2	2.4	4.64	0.78	5.00	4.24
Native America	1	1.2	3.14	-	0.00	0.00
Other	3	3.6	2.06	1.00	5.67	5.51
					POMS-D>20, *n*=10	
Herpes Viral Seroprevalence						
HSV-1	56	67.5				
HHV-6	59	71.7				
CMV	64	77.1				
EBV	56	67.5				

Note. *M*: Mean, *SD*: Standard Deviation, POMSD: Profile of Mood States-Depression, HSV-1: Herpes simplex virus type 1, HSV-2: Herpes simplex virus type 2, HHV-6: Human herpes virus-6, CMV: Cytomegalovirus, EBV: Epstein-Barr virus

HHV-6 seropositive titers, perceived stress and depressive symptoms. From independent sample t-tests analysis, the women with HHV-6 titers ≥ 6 (seropositive) women had a significantly lower PSS score than the women with HHV-6 titers < 6 (seronegative) (Table 9). From Pearson correlation analysis, the HHV-6 seropositive titers were negatively correlated with POMS-D score (r = -.130, p = .011) (Table 6).

When HHV-6 seropositive titers were entered in the regression analysis (R^2 =.332, adjusted R^2 = .325, F (4, 375) = 46.530, p = .000, R^2change = .001, F change (1, 375) = .304, p = .582), the HHV-6 seropositive titers (β = -.024, p = .582) were not associated with depressive symptoms. When dependent variable HHV-6 seropositive titers and independent variables PSS, race (African American) and number of children were entered in regression model (R^2=.033, F (3, 376) = 4.336, p = .005), the relationship between HHV-6 seropositive titers and PSS

(β = -.097, p = .063) was not significant.

CMV seropositive titers, perceived stress and depressive symptoms. From t-test analysis, there was no difference between POMS-D and PSS mean score in women with the CMV infection. When CMV seropositive titers were entered in the regression analysis (R^2 = .333, adjusted R^2 = .326, F (4, 375) = 46.751, p = .000, R^2change = .002, F change (1, 375) = .895, p = .343), the CMV seropositive titers (β = .042, p = .345) were not associated with depressive symptoms.

When dependent variable CMV seropositive titers and independent variables PSS, race (African American) and number of children were entered in regression model (R^2 = .088, F (3, 376) = 12.070, p = .000), the relationship between CMV seropositive titers and PSS (β = -.062, p = .220) was not significant.

CMV seropositive titers, perceived stress and fatigue symptoms. From the independent t-test results the women with CMV seroprevalence titers had a significant different POMS-Fatigue score (Table 9).

Perceived stress and fatigue symptoms. The perceived stress score was highly correlated with fatigue symptoms (POMS-F) (r = .426, p = .000) (Table 6). POMS-F score ranged from 0 to 25, mean score was 8.04. To explore the relationship between perceived stress and fatigue symptoms, supplementary hierarchical multiple regression was used in full sample (N = 380). Independent variables race (African American), and numbers of children were entered at Step 1, explaining 1.7 % of the variance in fatigue symptoms. After entry of the perceived stress in Step 2 the total variance explained by the model as a whole was 21.8% (R^2 = .218, adjusted R^2 = .212, F (3, 376) = 34.998, p = .000, R^2change = .201, F change (1, 375) = 96.713,

p = .000). The perceived stress explained the additional 20.1 % of the variance. The results suggest perceived stress (β = .459, p = .000) strongly predicts fatigue symptoms (Table 14).

Table 14
Relationship between Prenatal Perceived Stress and Fatigue Symptoms (N=380)

Variable	Step 1			Step 2		
	B	*SE B*	β	*B*	*SE B*	β
Intercept	8.098	.365		.909	.802	
AA	-1.579	.662	-.124**	-2.530	.599	-.199***
#Children	.339	.244	.072	.109	.219	.023
PSS				.346	.035	.459***
	R^2 = .017 Adjusted R^2 = .012 F(2, 377) = 3.302*			R^2 = .218 Adjusted R^2 = .212 F(3, 376) = 34.998***		
	R^2 change = .201 F change (1, 376) = 96.713***					

Note. AA: African American, #Children: Number of children, PSS: Perceived Stress Scale
*P <.05, **P < .01, ***P <.001

Perceived stress, depressive symptoms and fatigue symptoms. From Pearson correlation analysis, the depressive symptoms were highly correlated with fatigue symptoms (r = .546, p = .000). Hierarchical multiple regression was used to assess the ability of the prenatal perceived stress in predicting levels of fatigue symptoms after controlling for depressive symptoms, race (African American) and number of children. Independent variables race (African American), number of children, and depressive symptoms were entered at Step 1, explaining total of 33 % of the variance in fatigue symptoms. After entry of the perceived stress at Step 2 the total variance explained by the model as a whole was 35.5 %. The results suggest perceived stress (β = .198, p = .000) strongly predicts fatigue symptoms (Table 15).

Table 15
Predictors of Prenatal Fatigue Symptoms (N=380)

Variable	Step 1			Step 2		
	B	*SE B*	*β*	*B*	*SE B*	*β*
Intercept	4.641	.401		2.221	.744	
AA	-2.177	.549	-.171***	-2.470	.545	-.194***
#Children	.170	.202	.036	.104	.199	.022
POMS-D	6.529	.493	.563***	5.247	.588	.452***
PSS				.149	.039	.198***
	R^2 = .330			R^2 = .355		
	Adjusted R^2 = .324			Adjusted R^2 = .348		
	F(3, 376) =61.671***			F(4, 375) = 51.625***		
	F change (1, 375) = 14.729***					

Note. AA: African American, #children: Number of children,
POMS-D: Profile of Mood States-Depression, PSS: Perceived Stress Scale
*P <.05, **P < .01, ***P <.001

CMV seropositive titers and fatigue symptoms. The CMV seropositive titers (N = 222) were entered as a continuous variable in a separate regression analysis to examine whether women with higher CMV seropositive titers had more fatigue symptoms. The results revealed that CMV seropositive titers (β = .142, p = .009) significantly positively predicted fatigue symptoms after controlling for race (African American), number of children, perceived stress and depressive symptoms (R^2 = .374, adjusted R^2 = .360, F (5, 216) = 25.834, p = .000, F change (1, 216) = 6.963, p = .009) (Figure 8 & Table 16).

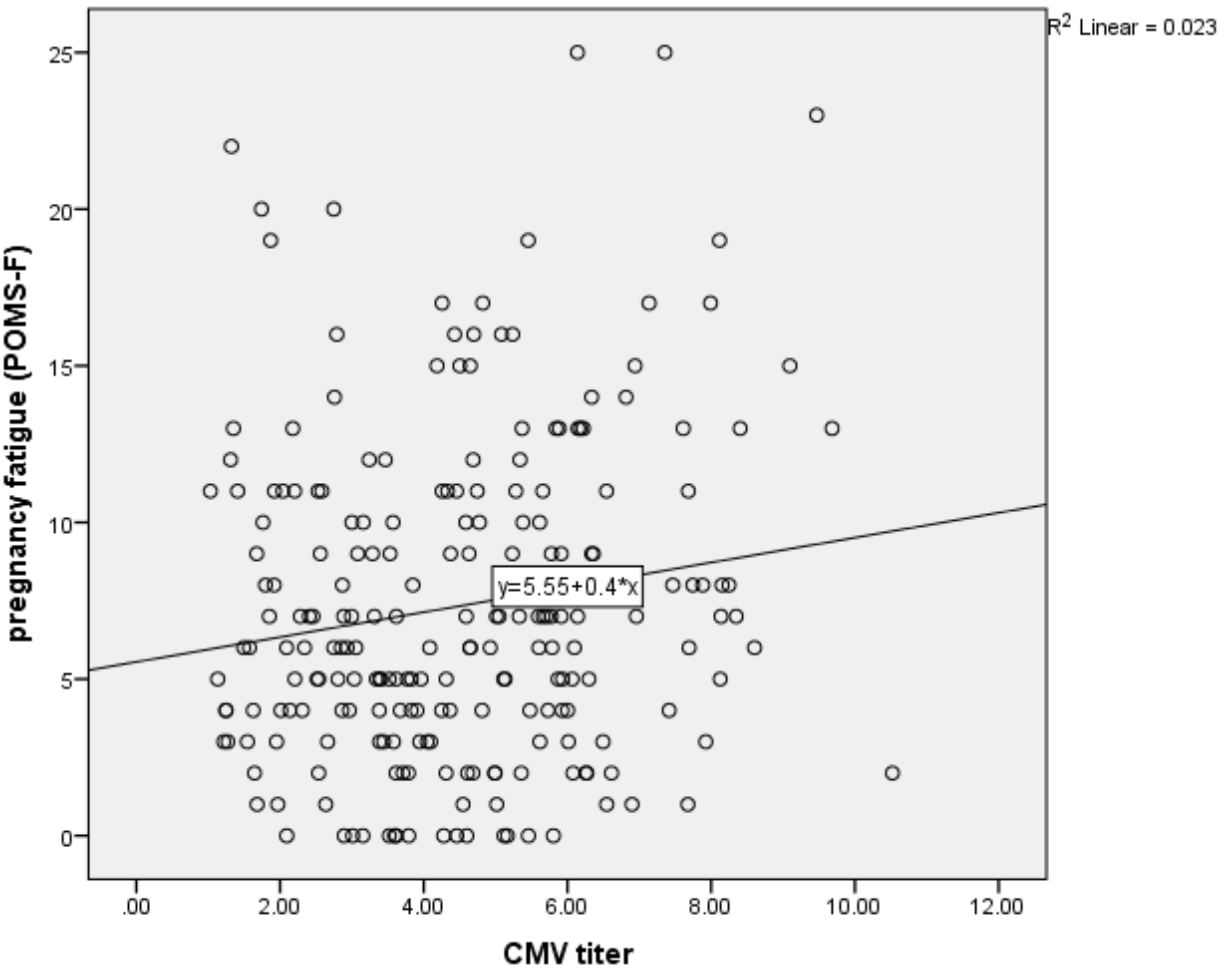


Figure 8. Scatter plot of women with Cytomegalovirus titers (CMV) (X-axis) and Profile of Mood States-Fatigue scores (POMS-F) (Y-axis) in seropositive women

Table 16
Predictors of Prenatal Fatigue Symptoms in CMV Seropositive Women (N= 222)

Variable	Step 1			Step 2		
	B	*SE B*	*β*	*B*	*SE B*	*β*
Intercept	1.753	.962		.104	1.136	
AA	-1.800	.633	-.162**	-1.796	.625	-.162**
#Children	.203	.241	.047	.182	.238	.042
PSS	.117	.051	.157*	.118	.050	.158*
POMS-D	5.678	.747	.497***	5.646	.737	.494***
CMV ≥1				.372	.141	.142**
	R^2 = .354 Adjusted R^2 = .342 *F*(4, 217) =29.734*** *F* change (1, 216) = 6.963**			R^2 = .374 Adjusted R^2 = .360 *F*(5, 216) = 25.834***		

Note. AA: African American, #children: Number of children, POMS-D: Profile of Mood States-Depression, PSS: Perceived Stress Scale, CMV: Cytomegalovirus
*P <.05, **P < .01, ***P <.001

Characteristics of women with CMV seropositive titers. Among the CMV seropositive women (*N* = 222), the age ranged from 17-45 years old. African American (32.3 %), Caucasian (32.3 %) and Hispanic (26.9 %) women had seroprevalence for the CMV infection. The CMV titers were not statistically different between races (*F* (5, 216) = .759, *p* = .580) or POMS-F scores from analysis of variance (ANOVA).The CMV seropositive women had significantly different POMS-D scores (*F* (5, 216) = 2.540, *p* = .029). Among the CMV seropositive women, 66.4 % also had the HSV-1 infection, 64.6 % had the EBV infection, 43.9 % had the HHV-6 infection and 28.7 % had the HSV-2 infection (Table 17).

Table 17
ANOVA of Sample Demographic Characteristics and Herpes Viral Titers in Women with CMV Seropositivity (N = 222)

Variable			CMV Titer		POMS-F Score		POMS-D Score	
	N	%	*M*	*SD*	*M*	*SD*	*M*	*SD*
Race								
Caucasian	71	32.0	4.24	2.08	7.65	4.75	3.13	5.21
African America	72	32.4	4.47	1.93	6.93	5.49	7.92	11.52
Hispanic	60	27.0	4.63	1.88	7.40	5.48	6.17	7.10
Asian	8	3.6	5.50	1.89	8.13	5.38	6.38	8.40
Native America	1	0.5	3.79	-	0.00	1.00	0.00	0.00
Other	10	4.5	4.20	2.49	7.40	4.99	7.00	5.98
			F (5, 216) = .759		*F* (5,216) = .568		*F* (5,216) = 2.540* POMS-D>20,*n*=10	
Herpes Viral Seroprevalence								
HSV-1	148	66.4						
HSV-2	64	28.7						
HHV-6	161	43.9						
EBV	143	64.6						

Note. *M*: Mean, *SD*: Standard Deviation, POMS-F: Profile of Mood States-Fatigue, POMS-D: Profile of Mood States-Depression, HSV-1: Herpes simplex virus type 1, HSV-2: Herpes simplex virus type 2, HHV-6: Human herpes virus-6, CMV: Cytomegalovirus, EBV: Epstein-Barr virus, *P <.05

Summary of hypothesis 2. Independent t-tests, Pearson correlation and regression analyses were performed on the separate herpes viral titers to explore the relationships among infection status, prenatal perceived stress, and depressive symptoms. Mediation analysis revealed that the herpes viral **(**HSV-2) seropositive titer was a mediator, partially mediating the effect of perceived stress on depressive symptoms. The HSV-2 seropositive titers were significantly positively associated with perceived stress and depressive symptoms in pregnant women; the women with higher perceived stress had higher HSV-2 seropositive titers and the women with higher HSV-2 seropositive titers had more depressive symptoms. African American women

(43.3 %) had higher depressive symptoms (POMS-D) scores and higher seroprevalence for the HSV-2 infection. Also, the women with CMV seropositive titers showed a positive association with prenatal fatigue symptoms after controlling for race (African American), number of children, perceived stress and depressive symptoms. However, the associations of perceived stress or depressive symptoms with HSV-1, HHV-6 and CMV seropositive titers were not statistically significant.

Hypothesis 3

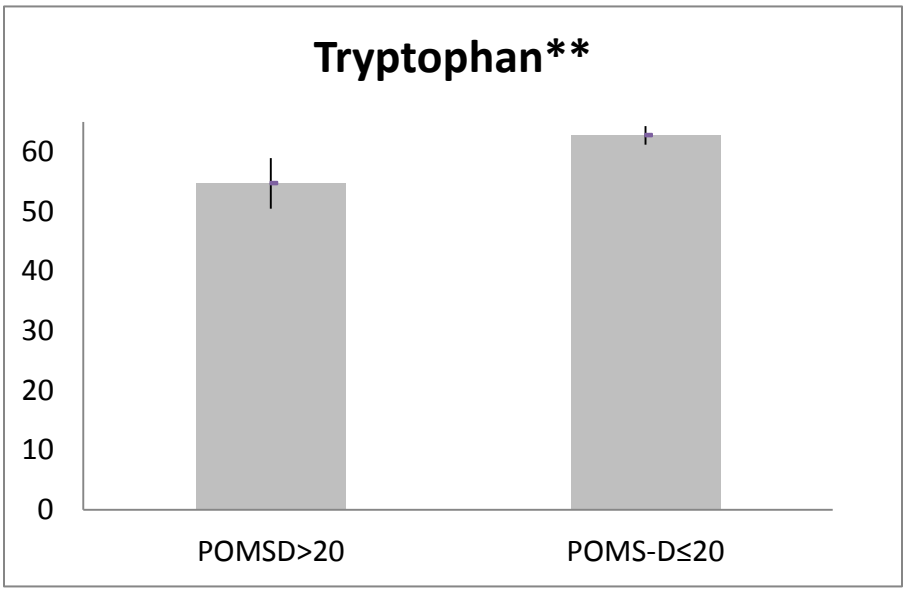
Kynurenine /tryptophan ratios, phenylalanine /tyrosine ratios, and neopterin levels are positively related to herpes viral titers in depressed pregnant women.

In this exploratory analysis, independent sample t-tests, Pearson correlation and multiple regressions were conducted to understand each herpes viral seroprevalence status in association with different biogenic amines and depressive symptoms.

The POMS-D (r = -.152, p = .003, two-tailed) and PSS scores (r = -.130, p = .011) were negatively correlated with phenylalanine. The following immune markers and amino acid levels were positively correlated: (1) nitrite with phenylalanine (r = .147, p = .004), (2) neopterin with phenylalanine (r = .159, p = .002), kynurenine (r = .355, p = .000), Phe /Tyr (r = .168, p = .001) and KYN /TRP ratios (r = .326, p = .000), (3) tryptophan with kynurenine (r = .384, p = .000), phenylalanine (r = .632, p = .000) and tyrosine (r = .543, p = .000), (4) phenylalanine with tyrosine (r = .692, p = .000).

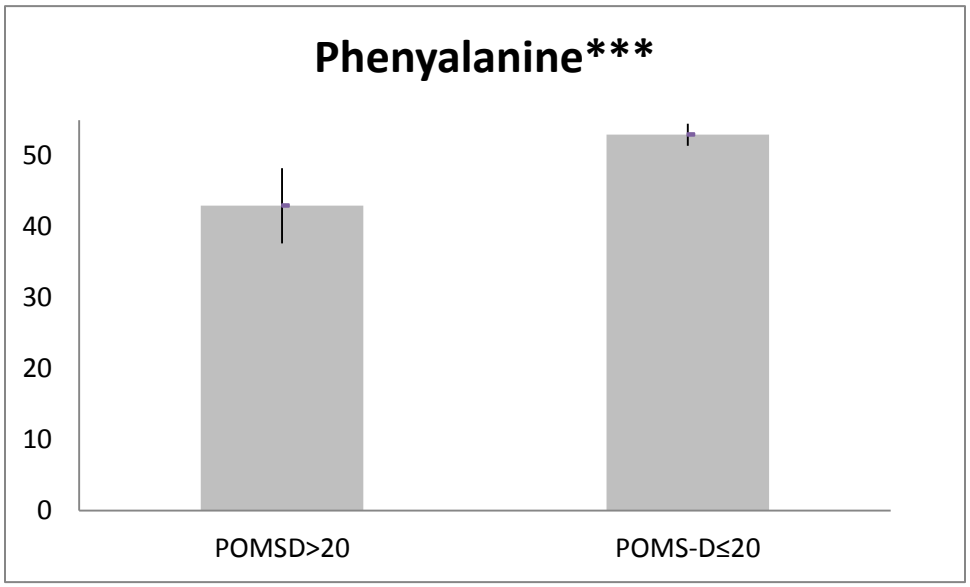
Negative correlation between biogenic amines as follow: (1) tryptophan with KYN/TRP ratios (r = -.237, p = .000), (2) KYN /TRP ratios with phenylalanine (r = -.163, p = .001) and tyrosine (r = -.263, p = .000) (Table 6).

POMS-D score > 20 group and biogenic amines. From t-test analysis, the women with POMS-D score > 20 group had significantly lower tryptophan and phenylalanine levels than the women with POMS-D score ≤ 20 group (Figure 9, 10 & Table 7).



**P <.01

Figure 9. Significantly lower tryptophan level in women Profile of Mood States- Depression (POMS-D) >20 group

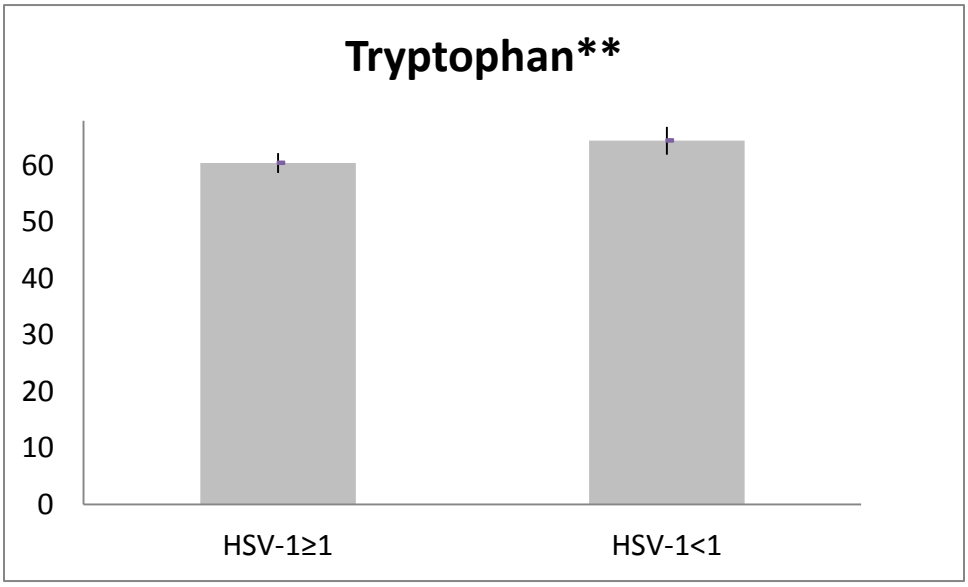


***P <.001

Figure 10. Significantly lower phenylalanine level in women Profile of Mood States- Depression (POMS-D) >20 group

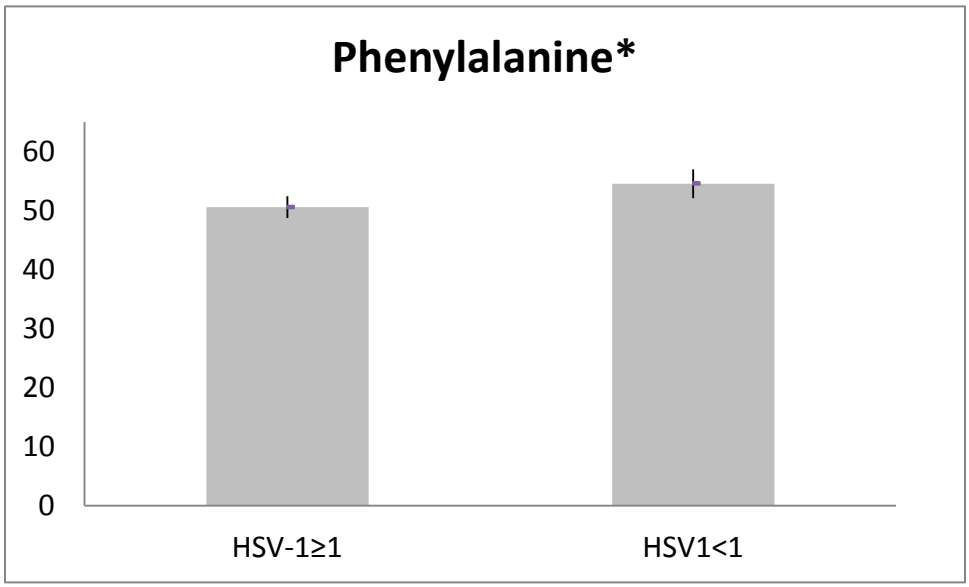
HSV-1 seroprevalence and biogenic amines. From t-test analysis, the women with HSV-1 seropositive titers had significantly lower tryptophan and phenylalanine levels than the women with HSV-1 seronegative titers (Figure 11, 12 & Table 18). From Pearson correlation analysis, HSV-1 titers were negatively correlated with tryptophan (r = -.152, p = .003) and phenylalanine (r = -.141, p = .006) (Table 6).

Multiple regression analyses were used to assess relationships between depressive symptoms and independent variables HSV-1 seropositive titers, and biogenic amines. When race (African American), number of children, HSV-1seropositive titers, and perceived stress were entered at Step 1, 33.2 % of variability in depressive symptoms was explained (F (4, 375) = 46.495, p = .000). Each biogenic amine was entered separately in Step 2. None of biogenic amines significantly influenced depression: for neopterin (β = .029, p = .508); for Phe /Tyr ratios (β = -.029, p = .662); for KYN /TRP ratios (β = -.033, p = .440).



**P <.01

Figure 11. Significantly lower tryptophan level in women with herpes simplex virus (HSV-1) seropositive titers



*P <.05

Figure 12. Significantly lower phenylalanine level in women with herpes simplex virus (HSV-1) seropositive titers

Table 18

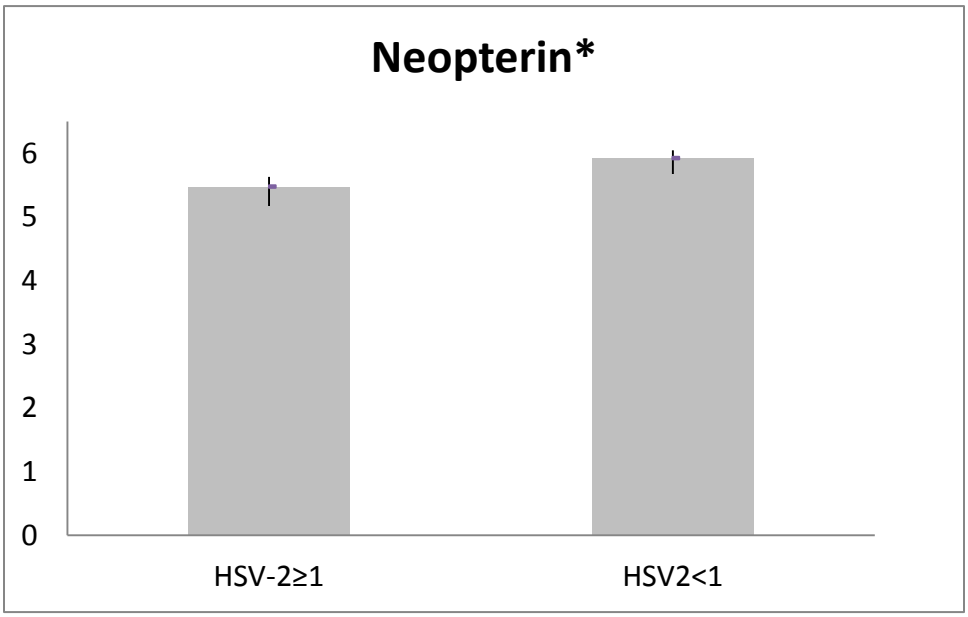
Summary Independent T-Test Biogenic Amines in HSV-1 Titers above and below Threshold (N = 380)

Variable	Viral Serology	*M*	*SD*	*t*	Mean difference	95% *CI*	η^2
Tryptophan	HSV-1 ≥ 1	60.51	13.29	-2.73**	-4.13	-7.10	.019
	< 1	64.63	16.17			to -1.07	
kynurenine	HSV-1 ≥ 1	1.88	.69	-.53			
	< 1	1.92	.81				
KYN / TRP	HSV-1 ≥ 1	31.89	11.60	1.53			
	< 1	30.06	11.31				
Neopterin	HSV-1 ≥ 1	5.78	1.95	-.50			
	< 1	5.87	2.21				
Nitrite	HSV-1 ≥ 1	19.79	14.34	1.07			
	< 1	18.26	13.17				
Tyrosine	HSV-1 ≥ 1	49.89	16.25	.47			
	< 1	51.71	17.90				
Phenylalanine	HSV-1 ≥ 1	50.57	14.00	-2.65**	-4.11	-7.16	.018
	< 1	54.18	16.15			to -1.06	
Phe /Tyr	HSV-1 ≥ 1	1.05	.23	-1.81			
	< 1	1.14	.70				

Note. KYN/TRP: Kynurenine/ Tryptophan, Phe/Tyr: Phenylalanine /Tyrosine, HSV-1: Herpes simplex virus type 1, *df* = 378, **P <.01, two-tailed.

HSV-2 seroprevalence and biogenic amines. From t-test analysis, the women with HSV-2 seropositive titers had significant lower neopterin level than the women with HSV-2 seronegative titers (Figure 13 & Table 19).

When race (African American), number of children, HSV-2 seropositive titers, and perceived stress were entered at Step 1, explaining 33.8 % of variability in depressive symptoms (F (4,375) = 46.868, p = .000). Each biogenic amine was entered separately in Step 2. None of biogenic amines significantly influenced depression: for neopterin (β = -.006, p = .885); for Phe /Tyr ratios (β = .030, p = .488); for KYN /TRP ratios (β = -.028, p = .506).



*P<.05

Figure 13. Significantly lower neopterin level in women with herpes simplex virus type 2 (HSV-2) seropositive titers

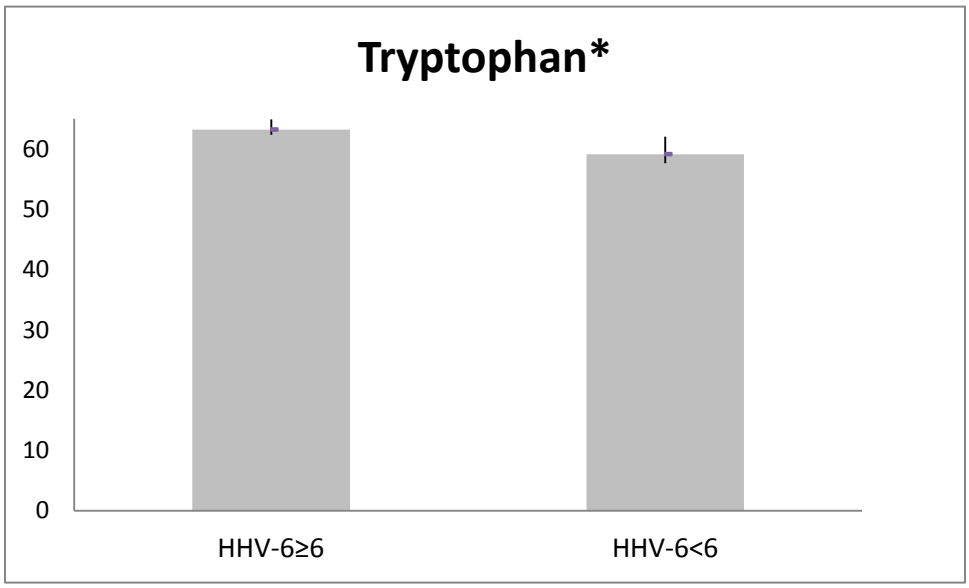
Table 19
Summary Independent T-Test Biogenic Amines in HSV-2 Titers above and below Threshold

Variable	Viral Serology	*M*	*SD*	*t*	Mean difference	95% *CI*	η^2
Tryptophan	HSV-2 ≥ 1	60.83	15.19	-1.01			
	< 1	62.68	14.59				
kynurenine	HSV-2 ≥ 1	1.83	.81	-.94			
	< 1	1.92	.72				
KYN /TRP	HSV-2 ≥ 1	30.47	11.79	-.62			
	< 1	31.28	11.43				
Neopterin	HSV-2 ≥ 1	5.48	1.41	-2.25*	-.45	-.85 to-.06	.015
	< 1	5.92	2.21				
Nitrite	HSV-2 ≥ 1	19.56	14.87	.32			
	< 1	19.01	13.58				
Tyrosine	HSV-2 ≥ 1	49.77	15.04	-.54			
	< 1	50.92	17.50				
Phenylalanine	HSV-2 ≥ 1	50.18	15.63	-.15			
	< 1	52.94	14.89				
Phe /Tyr	HSV-2 ≥ 1	1.03	.22	-1.27			
	< 1	1.10	.54				

Note. KYN /TRP: Kynurenine /Tryptophan, Phe /Tyr: Phenylalanine /Tyrosine, HSV-2: Herpes simplex virus type 2, *df* = 378 *P <.05, two-tailed.

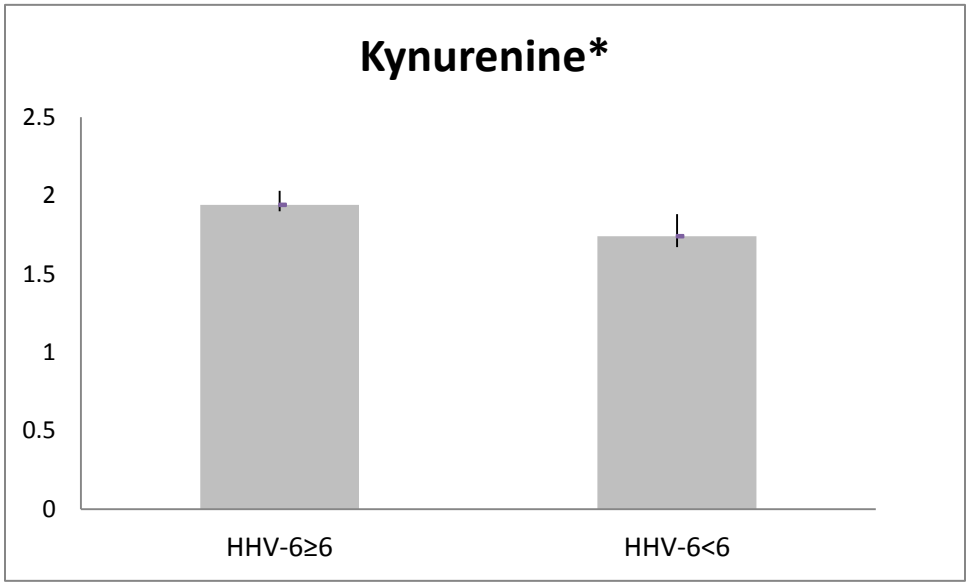
HHV-6 seroprevalence and biogenic amines. From t-test analysis, the women with HHV-6 seropositive titers had significant higher tryptophan, kynurenine and phenylalanine levels than the women with HHV-6 seronegative titers (Figure 14, 15, 16 & Table 20). From Pearson correlation analysis, HHV-6 titers were positively correlated with tryptophan (r = .158, p = .002), kynurenine (r = .127, p = .013) and phenylalanine (r = .163, p = .001) (Table 6).

Race (African American), number of children, HHV-6 seropositive titers, and perceived stress entered in the multiple regression at Step 1, explained 33.2 % of variability in depressive symptoms (F (4, 375) = 46.530, p = .000). Each biogenic amine was entered separately in Step 2. None of biogenic amines significantly influenced depression: for neopterin (β = -.012, p = .785); for Phe /Tyr ratios (β = .028, p = .513); for KYN /TRP ratios (β = -.030, p = .479).



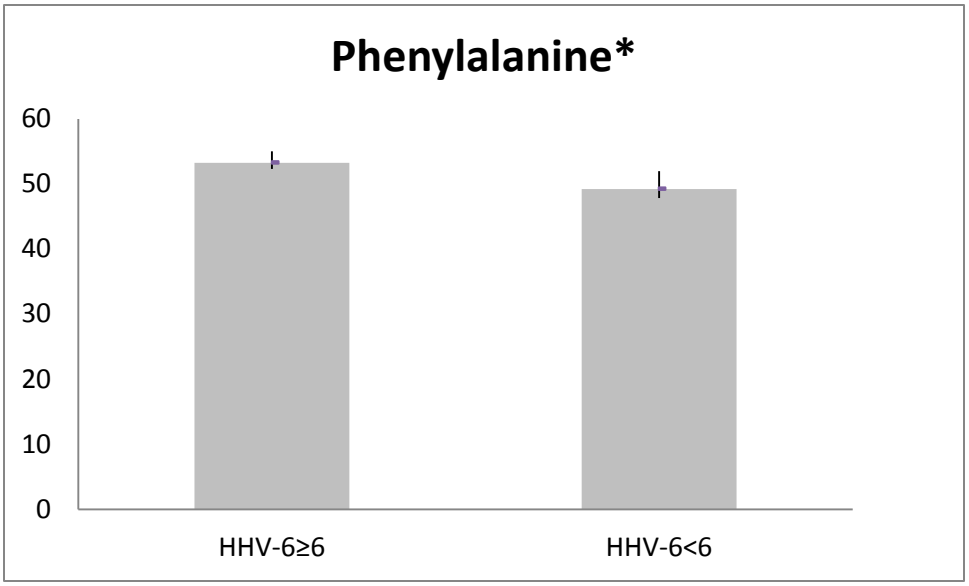
*P <.05

Figure 14. Significantly higher tryptophan level in women with human herpes virus-6 (HHV-6) seropositive titers



*P < .05

Figure 15. Significantly higher kynurenine level in women with human herpes virus-6 (HHV-6) seropositive titers



*$P < .05$

Figure 16. Significantly higher phenylalanine level in women with human herpes virus-6 (HHV-6) seropositive titers

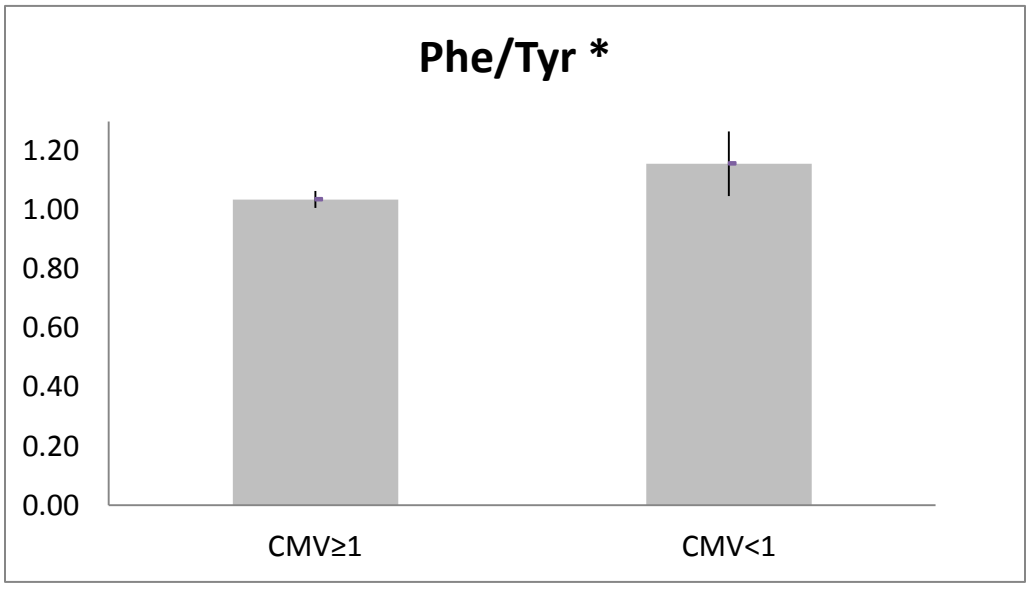
Table 20
Summary Independent T-Test Biogenic Amines in HHV-6 Titers above and below Threshold

Variable	Viral Serology	*M*	*SD*	*t*	Mean difference	95% *CI*	η^2
Tryptophan	HHV-6 ≥ 6	63.19	14.81	2.19*	3.89	.41 to 7.36	.013
	< 6	59.31	14.04				
kynurenine	HHV-6 ≥ 6	1.94	.76	2.12*	.19	.14 to .37	.013
	< 6	1.75	.69				
KYN /TRP	HHV-6 ≥ 6	31.38	11.55	.85			
	< 6	30.20	11.34				
Neopterin	HHV-6 ≥ 6	5.88	2.14	.89			
	< 6	5.97	1.82				
Nitrite	HHV-6 ≥ 6	18.91	14.27	-.56			
	< 6	19.84	12.43				
Tyrosine	HHV-6 ≥ 6	50.99	17.14	.65			
	< 6	49.65	16.51				
Phenylalanine	HHV-6 ≥ 6	53.30	15.46	2.24*	4.05	.50 to 7.61	.013
	< 6	49.24	13.40				
Phe /Tyr	HHV-6 ≥ 6	1.10	.54	1.27			
	< 6	1.03	.22				

Note. KYN /TRP: Kynurenine /Tryptophan, Phe /Tyr: Phenylalanine /Tyrosine, HHV-6: Human herpes virus-6, $df = 378$ *$P < .05$, two-tailed.

CMV seroprevalence and biogenic amines. From t-test analysis, the women with CMV seropositive titers had significant lower phenylalanine /tyrosine ratios than the women with CMV seronegative titers (Figure 17 & Table 21). From Pearson correlation analysis, CMV seropositive titers were negatively correlated with phenylalanine /tyrosine ratios (r = -.110, p = .032) and positively correlated with neopterin (r = .133, p = .009) (Table 6).

Multiple regression analyses were used with race (African American), number of children, CMV seropositive titers, and perceived stress entered at Step 1, explaining 33.3 % of variability in depressive symptoms (F (4, 375) = 46.751, p = .000). Each biogenic amine was entered separately in Step 2. None of biogenic amines significantly influenced depression: for neopterin (β = -.016, p = .704); for Phe /Tyr ratios (β = .033, p = .751); for KYN /TRP ratios (β = -.031, p = .472).



*P <.05

Figure 17. Significantly lower phenylalanine /tyrosine (Phe /Tyr) ratios in women with cytomegalovirus (CMV) seropositive titers

Table 21
Summary Independent T-Test Biogenic Amines in CMV Titers above and below Threshold

Variable	Viral Serology	*M*	*SD*	*t*	Mean difference	95% *CI*	η^2
Tryptophan	CMV ≥ 1	61.50	14.66	-1.22			
	< 1	63.37	14.79				
kynurenine	CMV ≥ 1	1.87	.74	-1.01			
	< 1	1.94	.75				
KYN /TRP	CMV ≥ 1	30.95	11.19	-.30			
	< 1	31.31	11.97				
Neopterin	CMV ≥ 1	5.94	2.21	1.19			
	< 1	5.68	1.82				
Nitrite	CMV ≥ 1	19.33	12.83	.33			
	< 1	18.85	15.21				
Tyrosine	CMV ≥ 1	51.02	16.79	.48			
	< 1	50.17	17.29				
Phenylalanine	CMV ≥ 1	51.22	14.79	-1.72			
	< 1	53.91	15.39				
Phe /Tyr	CMV ≥ 1	1.04	.22	-2.42*	-.12	-.22 to-.02	.015
	< 1	1.16	.71				

Note. KYN /TRP: Kynurenine /Tryptophan, Phe /Tyr: Phenylalanine /Tyrosine, CMV: Cytomegalovirus, *df* = 378, **P* < .05, two-tailed.

The separate hierarchical regression analysis was performed to assess the relationship between CMV seropositive titers (*N* = 222) and biogenic amine pathway. Independent variables neopterin, perceived stress, race (African American) and number of children were entered at Step 1 explaining 30.4 % of variance in the depression model (R^2 = .304, adjusted R^2 = .288, *F* (5, 216) = 18.898, *p* = .000). After entry of the Phe /Tyr ratios at Step 2 the Phe /Tyr ratios explained an additional 1.8 % of the variance in depressive symptoms (R^2 = .322, adjusted R^2 = .303, *F* (6, 215) = 17.038, *p* = .000, R^2change = .018, *F* change (1, 215) = .5.687, *p* = .018). The Phe /Tyr ratios (β = -.144, *p* = .018) were negatively associated with depressive symptoms (Figure 18 & Table 22). The CMV seropositive titers were not significantly associated with tryptophan, kynurenine and KYN /TRP ratios in the depression model.

Table 22
Predictors of Depressive Symptoms in CMV Seropositive Titers (N= 222)

	Step 1			Step 2		
Variable	*B*	*SE B*	*β*	*B*	*SE B*	*β*
Intercept	-.170	.121		.125	.172	
AA	-.027	.058	-.028	-.020	.057	-.020
#Children	-.021	.022	-.055	-.020	.022	-.052
PSS	.037	.004	.564***	.035	.004	.539***
CMV≥1	.005	.013	.020	.002	.013	.007
Neopterin	-.008	.012	-.037	.002	.012	.011
Phe /Tyr				-.298	.125	-.144*
	R^2 = .304			R^2 = .322		
	Adjusted R^2 = .288			Adjusted R^2 = .303		
	$F(5, 216) = 18.898$***			$F(6, 215) = 17.038$***		
	F change (1, 215) = 5.687*					

Note. AA: African American, #Children: Number of children, PSS: Perceived Stress Scale
KYN /TRP: Kynurenine / Tryptophan, Phe /Tyr: Phenylalanine /Tyrosine
CMV: Cytomegalovirus
*P <.05, ***P <.001

The associations of depressive symptoms or HSV-1, HSV-2, HHV-6 seropositive titers with biogenic amines were not statistically significant from the multiple regression analysis. Therefore, the path analysis initially planned in the dissertation proposal was excluded from the final hypothesis 3 analysis.

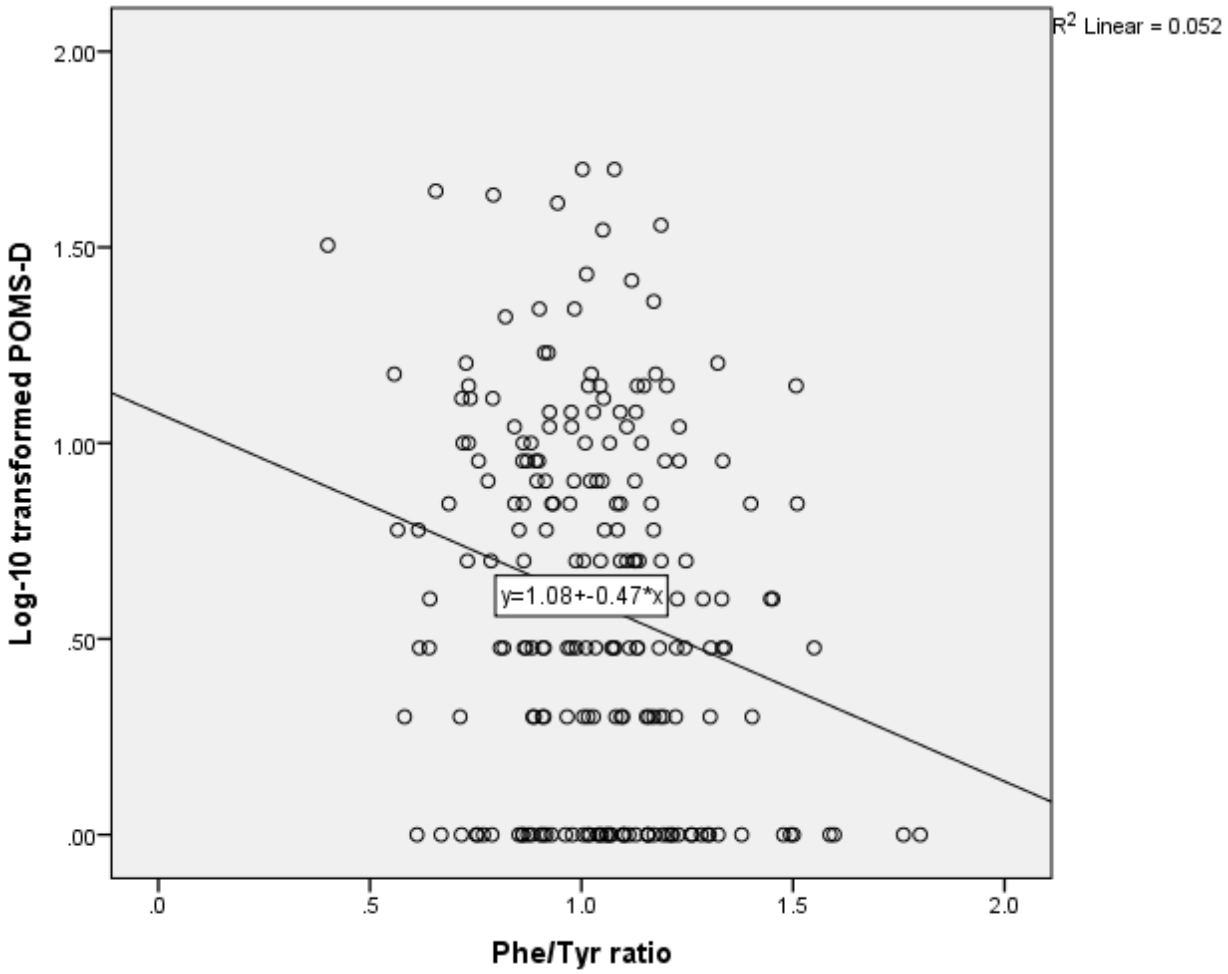


Figure 18. Scatter plot of Phenylalanine /Tyrosine (Phe /Tyr) ratios (X-axis) and log-10 transformed POMS-Depression scores (Y-axis) in cytomegalovirus (CMV) seropositive women

Summary of hypothesis 3. From the t-test analyses, the POMS-D score > 20 and HSV-1 seropositive group had significantly lower tryptophan and phenylalanine levels, the HSV-2 seropositive group had lower neopterin level, the HHV-6 seropositive group had higher tryptophan, kynurenine and phenylalanine levels and the CMV seropositive group had lower Phe /Tyr ratios.

From the Pearson correlation analyses, POMS-D and perceived stress scores were negatively correlated with phenylalanine levels. The following immune markers and amino acids

levels were positively correlated: (1) nitrite with phenylalanine, (2) neopterin with phenylalanine, kynurenine, Phe /Tyr and KYN/TRP ratios, (3) tryptophan with kynurenine, phenylalanine and tyrosine, and (4) phenylalanine with tyrosine.

Tryptophan concentrations were negatively correlated with KYN/TRP ratios and KYN /TRP ratios were negatively correlated with phenylalanine and tyrosine. Negative correlation between seropositive herpes viral titers and biogenic amines were as follows: HSV-1 with tryptophan and phenylalanine; CMV with Phe /Tyr ratios. Positive correlation between seropositive herpes viral titers and biogenic amines were as follows: HHV-6 with tryptophan, kynurenine and phenylalanine; CMV with neopterin.

The Phe /Tyr ratios were negatively correlated with depressive symptoms in women with CMV seropositive titers. However, there were no statistically significantly associations between the HSV-1, HSV-2, HHV-6 infection and the KYN /TRP or Phe /Tyr ratios from multiple regressions analysis.

CHAPTER FIVE

DISCUSSION

Hypothesis 1: Prenatal Stress and Depression

The national prevalence of depression in ages over 18 years is 16.6 % (Kessler et al., 2005). The prevalence of depression for women is between 6 % and 17 % (Kessler, 2003; Pratt, Xu, McQuillan & Robitz, 2012). The incidence of prenatal depression increases to 20 % during pregnancy (Bonari et al., 2004). In the current prenatal study, the clinical depression (POMS-D > 20) rate was 6.6 % compared to 12.7 % from Lancaster and colleagues review (2010).

The main finding from this study is that women with higher POMS-D scores had statistically significantly higher perceived stress; the perceived stress was a strong predictor of prenatal depressive symptoms. This relationship remained significant after controlling for race and number of children. This study sample (N = 380) provides robust evidence of the relationship between depression and perceived stress in pregnant women, comparable to Christian and colleagues (2009) who reported similar results with a small sample (N = 60).

In general, stress and depression scores are higher in low income and low education populations (Fagundes et al., 2012). In this study, women in the African American group had higher perceived stress and depression scores. Also, the more children pregnant women already had prior to being admitted to the study the more stress they had. These results reflect that pregnant women may lack social support or coping skills during stressful events. Similarly, Cassidy-Bushrow and colleagues (2012) reported that African American women have more depressive symptoms than non- African American in both non-pregnant and pregnant women.

Stress in early pregnancy has an extremely negative influence on psychological and physiological responses which may include depression, premature birth or pregnancy failure (Parker & Douglas, 2010; Coussons-Read, Okun & Nettles, 2007). Psychosocial stressors during pregnancy include daily hassles, job status, low income, low education, life experience, domestic violence and lack of social support (Lancaster et al., 2010). Wood and colleagues (2010) suggested that increased stress showed association with prenatal depressive symptoms. Escriba-Aguir and colleagues (2012) reported that poor social support or depression was a strong predictor of postpartum depression.

Hypothesis 2: Prenatal Perceived Stress, Herpes Viral Titers and Depression

Stress has been associated with a diminished T-cells response, such as reduction of cytokine production, memory T-cells and cytotoxic T-cells, which mediate killing herpes viral infection cells. Herpes viral IgG titers, a measure of latent herpes viral reactivation, are usually used in psychoneuroimmunology research. Elevated herpes viral IgG antibody titers reflect ineffective cellular immunity control over herpes viral reactivation (Glaser & Kiecolt-Glaser, 1994; Fagundes et al., 2012). T-cells respond to herpes viral reactivation, resulting in production of IFN-γ which mediates tryptophan depletion and causes depressive symptoms.

The important finding from this hypothesis testing was that the HSV-2 seropositive titers were associated with perceived stress and depressive symptoms in pregnant women; CMV seropositive titers were related to perceived stress and fatigue symptoms.

Herpes simplex virus (HSV). The HSV-1 seropositive rate was 57.8 % and the HSV-2 rate was 22 % in the current study. This result was similar to reports from prior studies: Straface and colleagues (2012) reported that pregnant women's HSV-2 infection rate was 22 % and Carter (2013) reported HSV-1 seroprevalence of 68 % of individuals aged over 12 in the United

States. The rate of co-infection with HSV-1 and HSV-2 in individuals aged over 12 was 16.6 % in the U.S. (Avgil & Ornoy, 2006).

The significant finding from the current study was that results were consistent when either using the full sample or the HSV-2 seropositive sample only, showing that HSV-2 seropositive titers were strongly associated with depressive symptoms. This relationship remained significant after controlling for race and number of children. Among only those with HSV-2 seropositive titers were entered in the multiple regression analysis, the women with higher perceived stress had higher HSV-2 seropositive titers which were strongly associated with depressive symptoms. The study findings were similar to Pratt and colleagues (2012), who reported that 19 % of people are HSV-2 seropositive and the depression rates in HSV-2 seropositive individuals was 7 % in general population of ages 20-59. People with HSV-2 infection were more likely to have depression.

Furthermore, this study found that African American women had higher HSV-2 seropositive rates and greater depressive symptoms than non-African American women. This result was similar to Gottlieb and colleagues (2002), who reported that black women had the highest overall HSV-2 seroprevalence in a study of five sexually transmitted-disease clinics. Also, this finding was similar to Borders and colleagues (2010) and Christian and colleagues (2012) who reported that African American women had higher perceived stress levels and herpes viral (EBV) titers in their studies. Races, culture, psychosocial status, biological factors (e.g. depression gene or epigenetic factors) or pathogenicity in different types of herpes virus may influence gene-gene or gene-environmental interaction and result in development of depressive symptoms.

Approximately 2-3 % of women had the primary HSV-2 genital herpes during pregnancy (Straface et al., 2012; Avgil & Ornoy, 2006). Two thirds of these pregnant women were asymptomatic (Avgil & Ornoy, 2006). Up to 90 % of HSV infection is undiagnosed and untreated (Perozzi et al., 2007; Pereira et al., 2003). Women usually feel psychological distress such as anger, denial, guilt, low self-esteem or depression after diagnosis with genital herpes.

Complications of HSV infection during pregnancy not only affect maternal health but also fetal and neonatal health. Nearly 85 % of HSV-2 transmission from mother to fetus occurs during delivery, while 5 % is caused by intrauterine infection. HSV-2 infection can cause miscarriage, stillbirth, congenital herpes and neonatal herpes infections for pregnant women with primary or recurrent HSV-2 infection (Straface et al., 2012; Corey & Wald, 2009; Perozzi et al., 2007; Avgil & Ornoy, 2006; Pereira et al., 2003).

Prenatal related depression is stated to be the leading cause of death during pregnancy. This study contributes to the limited literature on relationships between perceived stress, HSV-2 and depression among pregnant women. Mediation analysis revealed that HSV-2 was a mediator, partially mediating the effect of perceived stress on depressive symptoms. Prenatal perceived stress promoted HSV-2 reactivation and higher HSV-2 seropositive titers predicted prenatal depressive symptoms.

This study did not find a significant association between depressive symptoms and the prevalence of antibodies to other neurotropic herpes viruses, including HSV-1, HHV-6, CMV and EBV. This finding is consistent with findings of other studies (Christian et al., 2012; Amsterdam & Hernz, 1993).

Cytomegalovirus (CMV). Pregnant women infected with CMV can vertically transmit virus to fetus and cause the congenital CMV infection, hearing deficit, visual impairment or mental retardation (Walker, Palma-Dias, Wood, Shekleton & Giles, 2013). Women with a CMV primary infection during early pregnancy are at great risk for neonatal morbidity and mortality. Additionally, congenital CMV infection is a leading cause of neonatal morbidity. Women in the reproductive age group had seroprevalence rates ranging from 40 % to 83 % in the United States (Johnson & Anderson, 2013). In the present study, CMV seropositive rates were 60.1 %.

CMV has been indicated as a cell-mediated immune function marker and is linked to stress. In this study, there were no significant relationships among stress, CMV titers and depression in pregnant women. Pregnancy is an immunologically weak period and therefore pregnant women are vulnerable to herpes viral reactivation. Cancer patients and older adults show similar weakness in their immune system. The current study finding was similar to Fagundes and colleagues (2012) whose study reported that there were no associations between depression and CMV or EBV titers in women with breast cancer. Contrarily, Phillips and colleagues (2008) reported that higher CMV antibody titers were associated with depression and anxiety in older adults.

Further exploratory analysis showed that CMV seropositive titers were associated with fatigue symptoms (POMS-F). Separate Pearson correlation and regression analyses were conducted to examine relationships among perceived stress, depressive and fatigue symptoms in this study population. The results revealed that fatigue symptoms were correlated with depressive symptoms and perceived stress. Perceived stress strongly predicted fatigue symptoms after controlling for depressive symptoms, race and number of children in this population.

The important finding from this study was that CMV seropositive titers were significantly positively correlated with POMS-F score. Women with higher CMV seropositive titers had greater fatigue symptoms. This relationship remained strong after controlling for race, number of children, perceived stress and depressive symptoms. This prenatal study did not reveal significant relationships between fatigue and other herpes viral titers (HSV-1, HSV-2, HHV-6 or EBV).

Findings from this study are particularly exciting because they indicate a relationship between CMV seropositive titers and fatigue symptoms that has previously been unreported for a population of healthy pregnant women. Fatigue is a growing problem in public health. It has a negative impact on individual health and quality of life. It is estimated that 40 % of individuals complain of fatigue symptoms in primary care offices (Bower, 2012). Recently, the psychoneuroimmunology field has put emphasis on fatigue research to understand how neuro-immune system interactions affect fatigue symptoms.

Fagundes and colleagues (2012) reported that woman with breast cancer with higher CMV seropositive titers, but not the EBV titers, had greater fatigue symptoms. Other CMV related studies, such as Jaremka and colleagues (2013), reported that loneliness predicts pain, depression and fatigue which were related to CMV reactivation, not EBV. Although Fagundes (2012) and Jaremka (2013) reported CMV seropositive titers were related to fatigue symptoms, their studies only examined women with breast cancer not the general population of healthy pregnant women.

Bower and colleagues (2002) reported that people with increased persistent fatigue had higher levels of pro-inflammatory cytokine activity, such as increase IL-1 receptor antagonist (IL-1ra), soluble tumor necrosis factor receptor type II (sTNF-RII) and neopterin in cancer

patients. Bellmann-Weiler and colleagues (2008) reported that IFN-γ mediated tryptophan depleted in fatigue patients with EBV infection. Ross and colleagues (2009) reported that HIV positive pregnant women with more physical fatigue symptoms were associated with depression. Furthermore, chronic fatigue syndrome has been linked to herpes viral EBV infection (Bansal et al., 2012).

Bower (2012) proposed a model to demonstrate how inflammation and other factors influence fatigue symptoms. The model states that infection, radiation and cancer treatment triggers inflammation processing and induces fatigue symptoms. Other factors included in the fatigue model are genetic factors, neuroendocrine factors, psychosocial factors (e.g. stress and sleep), and immune factors (e.g. immune dysregulation or viral reactivation).

In conclusion, the current study found that prenatal perceived stress is related to CMV reactivation, which may involve neuroendocrine interactions different from those of other herpes viruses, and triggers fatigue symptoms. CMV seropositive titers play an important role in fatigue symptoms; therefore CMV could be an objective biomarker to assess fatigue symptoms in future study.

Human herpes virus-6 (HHV-6) and Epstein - Barr virus (EBV). In this study, the HHV-6 prevalence rate was 76.2 % and the EBV rate was 90.7 %. HHV-6 and EBV seropositive titers were unrelated to prenatal perceived stress or depressive symptoms, and thus HHV-6 and EBV are not discussed further.

Hypothesis 3: Perceived Stress, Herpes Viral Titers, Biogenic Amines, and Depressive Symptoms

Prenatal psychosocial stress and the normal physiology of pregnancy induce an immune shift from type 1 to type 2 which favors latent herpes viral reactivation. IDO activation and the inflammatory process during pregnancy reduce tryptophan availability and decrease viral replication. Consequently, serotonin synthesis decreases and dopamine synthesis from phenylalanine to tyrosine also decreases. Decreased tryptophan and serotonin or tyrosine and dopamine levels were related to prenatal depression (Scrandis et al., 2008).

In this study, POMS-D and perceived stress scores were negatively correlated with phenylalanine levels. The following immune markers and amino acid levels were positively correlated: (1) nitrite with phenylalanine, (2) neopterin with phenylalanine, kynurenine, Phe /Tyr and KYN /TRP ratios, (3) tryptophan with kynurenine, phenylalanine and tyrosine, and (4) phenylalanine with tyrosine. Tryptophan concentrations were negatively correlated with KYN/TRP ratios and KYN /TRP ratios were negatively correlated with phenylalanine and tyrosine. These results indicate that immune activation influences the amino acids metabolism.

Tryptophan and kynurenine. The independent t-test analysis in this study compared POMS-D scores > 20 depressed women to POMS-D scores ≤ 20 women indicated that POMS-D scores > 20 depressed women had a significantly lower tryptophan level. This finding was similar to that of Scrandis and colleagues (2008), who reported that prenatal depressive symptoms were negatively correlated with tryptophan levels.

Results from t-test analyses showed that the HSV-2 seropositive group had a significantly lower neopterin level which may indicate chronic inflammation (Capuron et al., 2011). The neopterin concentration is a marker for degree of immune activation (Murr, Widner, Wirleitner

& Fuchs, 2002). High neopterin level was associated with macrophage activation and inflammation (Sperner-unterweger, Kohl & Fuchs, 2012; Widner et al., 2002). Pearson correlation analysis showed negative correlations between seropositive herpes viral titers and biogenic amines: HSV-1 with tryptophan and phenylalanine; CMV with Phe /Tyr ratios; positive correlations between HHV-6 with tryptophan, kynurenine and phenylalanine.

The study results revealed that women with HSV-1 seropositive titers had lower tryptophan levels, but plasma kynurenine levels or KYN /TRP ratios did not increase. Women with HHV-6 seropositive titers had higher tryptophan and kynurenine levels. These results suggest that the IDO pathway may not activate in women with herpes viral infection or depressive symptoms. An alternative pathway may mediate tryptophan depletion, or dietary intakes may affect this metabolism pathway. This study finding was similar to Hughes and colleagues (2012), who reported no indication of kynurenine pathway activation in depressed patients.

Phenylalanine and tyrosine. This study also found that phenylalanine levels were lower in women with POMS-D > 20 and HSV-1 seropositive titers. Women with HHV-6 seropositive titers had higher phenylalanine levels. Phe /Tyr ratios were lower in women with CMV seropositive titers. Phenylalanine and the Phe /Tyr ratios were positively correlated with neopterin and nitrite was positively correlated with phenylalanine which suggests immune system was in activation status. The phenylalanine, Phe /Tyr ratios, and neopterin and nitrite findings may indicate that the immune system response to herpes virus infection varies with different status or through different metabolisms pathways.

CMV seropositive titers and Phe /Tyr ratios. Multiple regression analysis showed Phe /Tyr ratios were negatively correlated to depressive symptoms among women with CMV seropositive titers after controlling for race, number of children, perceived stress and neopterin. The study results were similar to Capuron and colleagues (2011) who reported a decrease in phenylalanine and tryptophan levels in elderly persons with depressive symptoms.

The Phe /Tyr ratio may indicate a dopamine synthesis biomarker (Neurauter et al., 2008). The dopamine precursor amino acid, tyrosine, is converted from phenylalanine. Basal ganglia neurotransmitter dopamine regulates a person's motivation and motor activity which are related to fatigue or depressive symptoms. Increased plasma Phe /Tyr ratio indicated a decrease in phenylalanine 4-hydroxylase (PAH) enzyme activity which might indicate increased oxidative stress and inflammation.

The study results indicated lower phenylalanine levels and Phe /Tyr ratios in women with the herpes viral infection. These results were different from other literature. For example, Felger and colleagues (2013) reported that plasma Phe /Tyr ratios were significantly increased in hepatitis C patients with IFN-α treatment. Zangerle and colleagues (2010) reported that blood Phe /Tyr ratios were increased in patients with HIV-1 infection.

The difference in these results could be due to only using a single blood sample test which makes it difficult to assess inflammation and metabolism status or alternative pathways between PAH to tyrosine or tyrosine to L-dopa activity. This altered biosynthesis processing of 5, 6, 7, 8-tetrahydrobiopterin (BH4) transfers hydrogen atoms to molecular oxygen and then to quinonoid 7, 8-dihydrobiopterin (BH2) (Neurauter et al., 2008). BH4 is an enzyme cofactor for formation of monoamine neurotransmitters serotonin, dopamine, norepinephrine and epinephrine. BH4 also shared a second pathway with folate (Miller, 2008; Neurauter et al.,

2008). Thus, nutrition supplement of folate or vitamin B12 and antioxidant vitamins C and E may influence this pathway during pregnancy.

Limitations of the Study

There are several limitations in this study:

First, this is a cross-sectional study with only a single blood sample test which limits the ability to draw conclusions about the direction of causality. A longitudinal study is needed to carefully describe herpes viral reactivation and investigate relationships between herpes viral reactivation and the IDO pathway in prenatal depression.

Second, the conceptual framework and hypothesis of the current study were based on a review of existing literature. The growing literature supports a role for stress induced immune shifting from Th1 to Th2 immunity. The immune effect theoretically reactivated the latent herpes virus and caused an imbalance of neurotransmitters. The current study outcome is that the herpes virus (HSV-2) was a mediator and had a partially mediating effect on the influence of perceived stress on depressive symptoms in pregnant women. The outcome of the study supports the proposed conceptual frame work and hypothesis. A reversed causality is possible in which depressive symptoms induce herpes viral reactivation. Future research is needed to explore different pathways among stress, herpes viral reactivation and depressive symptoms.

Third, the study data set was limited to a total of 380 out of the 631 pregnant women recruited from the original R01 study. The HSV-2 seropositive sample included only 83 cases. Future study may recruit more HSV-2 seropositive women.

Fourth, the POMS-D was a self- report questionnaire to measure mood status from the previous week. The POMS-D questionnaire is a general screening tool for clinical depressive symptoms. Different tools to repeat mood distress assessment during pregnancy are needed; such

as the Edinburgh Postnatal Depression Scale (EPDS), the Patient Health Questionnaire-9 (PHQ-9), the Beck Depression Inventory (BDI), and the Epidemiological Studies Depression Scale (CES-D) (Nylen et al., 2013).

Fifth, some data on the women with depressive symptoms (POMS-D > 20) and herpes viral seropositivity were not available to analyze; for example, demographics data, sleep pattern, nutrition and clinical assessment. More socioeconomic and clinical assessments data would be needed for future study to improve identification of potential risk factors of prenatal depression.

Sixth, immune status data (e.g. cytokines, cortisol and other biomarkers) and herpes viral seroprevalence (e.g. primary or recurrence) were limited from R01 study. Pregnant women's immune status and the herpes viral status should be included in future studies to better understand mechanisms of the relationships among prenatal stress, herpes viral reactivation and depression.

Finally, psychosocial stress has been found to be a significant predictor of depression and fatigue among herpes viral seropositive individuals. Therefore, to identify psychosocial stress, screening of herpes viral seroprevalence should be included and tools to assess fatigue symptoms are needed in future research.

Implications for Future Research

The outcome of this study is significant for practicing clinicians because they will be able to identify risk factors (demographic, psychosocial, herpes viral titers, biomarkers) and assess potential prenatal depression or potential postpartum depression and herpes viral infection during routine obstetric care. However, there are still gaps in knowledge that need to be addressed.

Screening tests to prevent the maternal herpes viral infection or depression are very important in public health, not only in maternal health but also in neonatal health. There is

insufficient data to support a recommendation screening for depression or routine herpes viruses (HSV-1, HSV-2, HHV-6, CMV, and EBV) in current clinical obstetric care. It is important to detect and prevent fetal damage and long-term adverse outcomes for subclinical state viral infection during pregnancy.

The study results provide an opportunity for important future research. Future research will continue to provide clinicians with a precise outline of how prenatal stress and psychosocial factors play a role in immune activation, promote herpes viral reactivation, and lead to depression or adverse pregnancy outcomes such as preterm delivery.

Future research could be done with longitudinal studies over different trimesters on maternal psychosocial risk factors, physical wellbeing, and pro-inflammatory biomarkers such as viral DNA. Maternal-neonatal outcomes data collection could add great value for future study**.** Moreover, based on results of this study, HSV-2 and CMV seropositive titers could be the objective biomarkers to assess depression and fatigue symptoms in future research.

The pregnant women were not informed of their own herpes viral infection status, or possibility of recurrence and methods of prevention efforts in the current study. Further research data will reinforce the need for proactive stress reduction through healthy behaviors such as sleep, exercise, and stopping smoking. Clinicians can provide better education to patients to encourage these behaviors. These could lead to reduced herpes virus recurrence rates and prevention of prenatal depression and neonatal herpes viral infection.

The current study outcome also builds a foundation in future psychoneuroimmunology research on stress, depression and herpes viral infection among different populations with immunosuppression such as elderly adults, caregivers, and individuals with HIV/AIDS, autoimmunity or organ transplantation.

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APPENDICES

Appendix A: **Demographic Form**

Last Name:	**First Name:**	**Initial:**

DOB: **_____/_____/_____** **Due Date:____/____/____** **_** **Pre-Pregnancy Wt:_____** **Today's Weight:** **_____**	**Address:** **Street:_____** **____** **City:_____** **State:_____** **Zip Code: _____** **Ht: _____ BMI:_____**	**Phone Number:** **_____** **Home:_____** **_____** **Cell:** **_____** **Emergency Contact Name:_____** **Phone Number:_____** **Email:_____** **__**

Race/Ethnicity:	**Education:**	**Household Income (Yearly):**
○ Caucasian ○ African-American ○ Asian/Pacific Islander ○ Native American ○ Hispanic Origin ○ White ○ Black ○ Other ○ Country of birth : _____ ○ Father of the baby ethnicity _____	○ Grammar School ○ Middle School ○ High School Graduate ○ College Graduate ○ Post Graduate ○ Type of work_____ ____	○ Under $4,999 ○ $5,000-14,999 ○ $15,000-24,999 ○ $25,000-39,999 ○ $40,000-69,999 ○ $70,000+

Marital Status:	**Current Working Status:**	**Number of Pregnancies:**	**How many children do you have?**	**Number living in household:**
○ Single ○ Married ○ Divorced ○ Widowed	○ **Part time** ○ **Full time** ○ **Not at all** **Hours/week:___** **___**	**___ Total pregnancies** **___ Full Term** **___ Premature** **___ AB, induced** **___ AB, Spontaneous** **___ Ectopics** **___ Multiple Births** **___ Living**	**_____** **__**	**_____**

Do you Smoke? ○ Yes ○ No If Yes, how many cigarettes/day:_____	**Did you smoke during your pregnancy?** ○ Yes ○ No If Yes, how many cigarettes/day:_____	**Do you drink alcohol?** o None ○ 1 drink / week ○ 2-3 drinks / week ○ More than 2-3 drinks /week	

Are you currently receiving any medical treatment for any health problems? If so plea

- ○ No
- ○ Yes, please list

1.

2.

3.

Are you taking any prescription edications?

- ○ No
- ○ Yes, please list

1.

2.

3.

Have you ever had severe eye inflammation? When?

o No

o Yes _____

Have you ever owned a cat? When?

o No

o Yes _____

Do you own a cat now?

o No

o Yes

Do you ever eat very rare or raw meat?

o No

o Yes

How many years have you lived in the U.S.? _____

What other countries have you lived in? How long for each country?

_____ _____

_____ _____

_____ _____

_____ _____

Have you ever been diagnosed with Toxoplasmosis?

o No

o Yes _____ When? _____ **What were your symptoms?** **_____ _____** **_____ _____**	

Appendix B: Perceived Stress Scale

Stress Since We Last Visited with You

Study ID #: _____ Date: _____

The questions in this scale ask you about your feelings and thoughts since we last visited with you. In each case, you will be asked to indicate how often you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer each question fairly quickly. That is, don't try to count up the number of times you felt a particular way, but rather make a reasonable estimate.

Please check the correct answer:

1 = Never 2 = Almost never 3 = Sometimes 4 = Fairly often 5 = Very often

	1	2	3	4	5
How often have you been upset because of something that happened unexpectedly?					
How often have you felt that you were unable to control the important things in your life?					
How often have you felt nervous and "stressed"?					
How often have you dealt successfully with irritating life hassles?					
How often have you felt that you were effectively coping with important changes that were occurring in your life?					
How often have you felt confident about your ability to handle your personal problems?					
How often have you felt that things were going your way?					
How often have you found that you could not cope with all the things that you had to do?					
How often have you been able to control irritations in your life?					
How often have you felt that you were on top of things?					
How often have you been angered because of things that happened that were outside of your control?					
How often have you found yourself thinking about things that you have to accomplish?					
How often have you been able to control the way you spend your time?					
How often have you felt difficulties were piling up so high that you could not overcome them?					

Appendix C: Copyright Approval Letter

USF HEALTH

November 6, 2013

Dear Dr. Hsu,

By this letter I am giving you permission to publish the demographic form that was used in the parent study of your dissertation, "Influence of lactation on postpartum stress and immunity". I am also verifying by this letter that permission to use the electronic form of the Profile of Mood States (POMS) used in the parent study was purchased from Multi-Health Systems, Inc, in 2007.

Sincerely yours,

Maureen W. Groer

Maureen W. Groer, RN, PhD

Gordon Keller Professor

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Appendix D: IRB Approval Letter

USF
UNIVERSITY OF
SOUTH FLORIDA

RESEARCH INTEGRITY AND COMPLIANCE
Institutional Review Boards, FWA No. 00001669
12901 Bruce B. Downs Blvd., MDC035 • Tampa, FL 33612-4799
(813)974-5638 • FAX(813)974-7091

3/22/2013

Maureen Groer, RN, Ph.D.
University of South Florida
College of Nursing
12910 Bruce B Downs Blvd
MDN 22
Tampa, FL 33612

RE: **Expedited Approval for Amendment**
IRB#: Ame3_104998
Title: Screening for Thyroid Disease

Dear Dr. Groer:

On 3/21/2013, the Institutional Review Board (IRB) reviewed and **APPROVED** your Amendment. The submitted request has been approved for the following:

1. Pao-Chu Hsu is being added to the study. She will be using data from the study for her dissertation.
2. Hsu Dissertation.

Approved Item(s):
Protocol Document(s):
Hsu Dissertation

We appreciate your dedication to the ethical conduct of human subject research at the University of South Florida and your continued commitment to human research protections. If you have any questions regarding this matter, please call 813-974-5638.

Sincerely,

Janelle Perkins, Pharm.D.

Janelle Perkins, Pharm.D., Chairperson
USF Institutional Review Board

ABOUT THE AUTHOR

Pao-Chu Hsu was born and grew up in Taipei, Taiwan. She earned an A.S. in Nursing, a B.S. in Biology Science and an M.S. in Microbiology and Immunology in Taiwan. She taught Microbiology and Immunology courses at the Junior College of Nursing and Junior College of Medical Technology before coming to the United States.

She also earned an M.S. in Nursing from the Family Nurse Practitioner program at the University of South Florida (USF) in 2004. She practiced as a certified family nurse practitioner at a primary care office and various long-term rehabilitation facilities from 2004 to 2007. She joined the Infectious Disease division of the College of Medicine at USF in 2007 and practiced as an Infectious Disease nurse practitioner at Tampa General Hospital in Florida until 2012. She was an instructor at USF College of Nursing in 2012. She received a Ph.D. in Nursing Science also from USF in 2013. She currently practices as a family nurse practitioner in the primary care office setting.