THE EVALUATION OF HUMAN HERPESVIRUS 8 INFECTION AND BENIGN PROSTATIC HYPERPLASIA IN TOBAGO

by

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University of Pittsburgh, 2014

ABSTRACT

Benign prostatic hyperplasia (BPH) is a disease which carries a high public health burden as it imposes a decreased quality of life and affects an increasing proportion of men as they age. It is known that BPH represents a chronic inflammatory state. Understanding underlying factors which drive the etiology of BPH is of utmost importance. One possible causative factor which has been considered is sexually transmitted infections. An association between seroprevalence of human herpesvirus 8 (HHV-8), a sexually transmitted infection, and increased risk of prostate cancer among a cohort of men in the island of Tobago has been reported. This study aims to further examine a potential association between HHV-8 seroprevalence and development of BPH through contribution to a chronic inflammatory state. The HHV-8 seroprevalence was determined among 168 men classified as having BPH (elevated serum prostate specific antigen levels ≥4.0ng/ml and cancer-free biopsy) and compared to 234 controls (men with normal prostates as defined by a PSA <4.0 ng/ml and a normal digital rectal exam) utilizing an enhanced immunofluorescence assay. Circulating pro-inflammatory cytokine levels were compared in a subset of both groups (120 BPH cases and 87 controls) utilizing the Meso Scale Discovery immunoassay.

A significant difference in percentage of HHV-8 seropositive individuals was observed with BPH compared to controls (p=0.000). A significant difference was also found between all abnormal inflammatory prostate conditions (BPH and prostate cancer as compared to controls

($p=0.000$). Significant differences were observed in mean levels of a majority of serum cytokines examined between individuals in BPH case and control groups ($p<0.05$). Comparison of BPH and control groups stratified by infection demonstrated significant differences in mean serum levels of a majority of pro-inflammatory cytokines which were examined. This study was the first of its kind to demonstrate a potential association between HHV-8 and BPH, and provided preliminary evidence to support increased pro-inflammatory response to BPH disease and HHV-8 viral infection. Further studies are needed to strengthen this association and provide further evidence of an inflammatory cytokine response.

Statement of Public Health Relevance: BPH is a disease which affects a significant proportion of the men with an estimated prevalence of 90% by the age of 70. This high prevalence places a significant burden on the healthcare system, driving up health care costs. Additionally, this disease can lead to loss of quality of life. Understanding underlying risk factors including infectious co-factors including those of infective origin will allow healthcare professionals take necessary interventions to reduce incidence and ultimately public health burden.

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1.0 INTRODUCTION

1.1 HUMAN HERPESVIRUS 8

Human herpesvirus 8 (HHV-8) also known as Kaposi's sarcoma-associated herpesvirus is the etiologic agent of many cancers including Kaposi's sarcoma (KS) [1, 2]. HHV-8 was discovered in 1994 by Chang and Moore in tissue from AIDS patients with KS lesions [3]. HHV-8 is classified as a gamma-2 herpesvirus with a characteristic double-stranded linear DNA structure. The genome is 165 kb in size composed of 85 open reading frames (ORFs). These ORFs allow the virus to transcribe proteins necessary for replication, latency, and modulation of the immune system [4].

1.1.1 HHV-8 and Cancer

HHV-8 is associated with several cancers including KS, primary effusion lymphoma (PEL), and some forms of multicentric Castleman's disease. In 2010, HHV-8 was identified as a group 1 carcinogen by the International Agency for Research on Cancer (IARC) due to its specific public health threat related to its oncogenic potential [5].

1.1.1.1 Kaposi's Sarcoma

KS manifests itself as raised lesions or nodules which are either red, purple, brown, black in color, found on various tissues including skin, mouth, gastrointestinal and respiratory tract [6]. The disease was first reported in patients as early as 1872 by Moritz Kaposi, a Hungarian Physician who observed the tumor and described it as an "idiopathic multiple pigmented

sarcoma of the skin [7].” The disease was characterized as an angioproliferative disorder in older men. Later, reports surfaced in 1972 of herpesvirus particles being isolated from KS lesions through electron microscopy [8]. In 1994, HHV-8 viral DNA was isolated in a lesion from a patient with AIDS [9]. KS lesions have a characteristic shape morphology with infected cells having a spindle shape [10, 11]. The incidence of KS varies widely. In the general population, the incidence is 1:100,000, while in the HIV infected individuals, its incidence increases significantly to 1:20 [8, 11]. Diagnosis of KS can be confirmed only by biopsy of suspected lesion and microscopic diagnosis with detection of latent associated nuclear antigen (LANA) [4].

There are multiple variants of KS disease. These include Classical KS, AIDS-associated KS, endemic KS, and iatrogenic KS. In all four forms, reactivation of the virus due to some form of immune suppression, enables development of KS. Classical KS is consistent with the angioproliferative disorder first described by Moritz Kaposi. The disease occurs in older men of primarily Mediterranean descent, European Jewish ancestry, as well as eastern African ancestry. The disease occurs due to age related immune weakening [12, 13]. AIDS-associated KS is present in HIV-infected individuals, primarily males [4, 14]. It is one of the commonest forms of KS, and one of the commonest diseases in those with AIDS. Endemic KS is a form of disease primarily prevalent in Africa [15-18]. Immune suppression through HIV allows for the propagation and increased prevalence of KS [18]. In this form of KS it is notable that younger populations are affected [17-18]. Lastly, Iatrogenic KS, occurs in organ transplant patients. In this form of KS, immunosuppression due to the transplantation allows reactivation of HHV-8 leading to disease [20].

1.1.1.2 Primary Effusion Lymphoma (PEL)

HHV-8 is the etiologic agent of PEL. PEL is a rare B cell non-Hodgkin's lymphoma with a prevalence of 4% overall and a 5-20% prevalence in HIV/AIDS patients [21-23]. PEL mainly occurs in body cavities, such as pericardium, pleural space, and peritoneum. Prognosis for patients is poor with median survival time of six months. Diagnosis of PEL includes confirmation of HHV-8 infection in addition to examination of suspected tissue [23]

1.1.1.3 Multicentric Castleman's Disease

MCD is a disease of lymphoid tissue which progresses in a manner consistent with lymphomas. MCD is not classified as a cancer rather a lymphoproliferative disorder, where there is hyperplasia of the tissue. However, the disease is highly similar to lymphoma and patients often progress on to develop lymphomas. Treatment is the same as with other cancers, which is treatment with chemotherapy and/or radiation therapy. The disease is sometimes found in patients co-infected with HIV [24].

1.1.2 Tropism and Viral Entry

HHV-8 infects a number of cell types in vivo. These include endothelial cells, dendritic cells, activated B cells, and activated macrophages. While these cell types can be infected with HHV-8 only productive infection has been reported in B cells [25, 26]. Infection requires successful entry into cells. Successful entry is accomplished by a binding receptor, heparan sulfate as well as an entry receptor which varies depending on cell type [4, 27]. Entry receptors that have been identified include dendritic cell-specific ICAM-3 grabbing nonintegrin (DC-SIGN), which is expressed on dendritic cells, activated B-cells and macrophages, amino acid

transporter protein (xCT), α3β1 integrin receptor, and the ephrin receptor A2 [28]. The binding receptor is used to aggregate virus particles to the cell surface and the entry receptor allows viral entry through means of endocytosis, or fusion of the viral envelope.

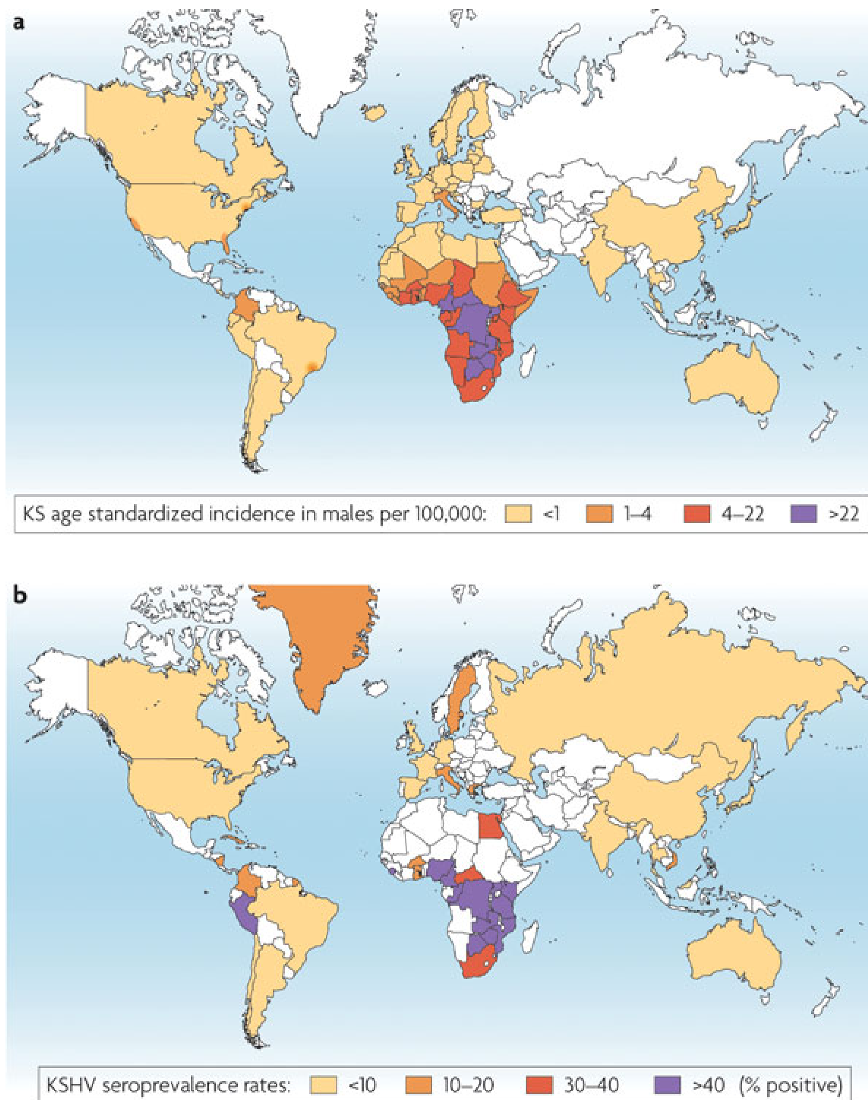
1.1.3 Epidemiology of HHV-8

1.1.3.1 Virus Transmission

Transmission of the virus occurs through direct sexual contact as well as non-sexual contact. Transmission through salivary exchange is believed to be the means of non-sexual contact. The importance of non-sexual contact through saliva cannot be understated [8, 29, 30]. In Africa, childhood KS is highly prevalent, and it is believed that the practice of pre-mastication of food by the mother leads to high rate of mother to child transmission [31-36]. Laboratory evidence suggests that that the HHV-8 virus readily replicates *in-vitro* in primary oral-derived epithelial cells [8, 36]. HHV-8 can enter lifelong latent infection evading immune responses, and reactivate into a lytic infection state [4]. This occurs more often in the immunocompromised populations. Healthy individuals are frequently unaffected by primary infections, and in worst case may experience diarrhea, fatigue, and rash if viral reactivation occurs [75]. This is in contrast where reactivation of the virus in an immunocompromised individual can lead to severe disease. While disease symptomology is likely to be experienced when virus is in a lytic state, both states are important in development of HHV- 8 associated cancers [4, 37].

1.1.3.2 Seroprevalence

The seroprevalence of HHV-8 varies depending on geographical region. The seroprevalence is less than 10% in northern Europe, Asia, and the United States with an estimated 1-3% seroprevalence in blood donors [8]. This increases to about 10-30% in the Mediterranean region, and significantly increases to 30-50% in sub-Saharan Africa. In the Caribbean, seroprevalence is estimated to be between 10-20% [8] (Figure 1).



Nature Reviews | Cancer

Figure reprinted with permission from *Nature Reviews Cancer*, *Mesri et al.,* Age-standardized incidence in males per 100,000 and HHV-8 seroprevalence rates demonstrate a significantly high burden in sub-Saharan Africa compared to other geographical areas.

Figure 1. Incidence of Kaposi's sarcoma and Seroprevalence rates of HHV-8

1.2 INFLAMMATION

Inflammation is part of a successful response to bodily injury and/or infection. When properly regulated, inflammation serves an effective means to aid in recovery and return to homeostasis. However, there are consequences for uncontrolled inflammation. Many substances including cytokines, chemokines, growth factors, reactive oxygen and nitrogen species are all continually in a state of increased production. All of these factors can have detrimental effect in the processes of cell proliferation, cell cycle control, and apoptosis [38]. A number of diseases are thought to have increased inflammation as an underlying factor. These include benign conditions such as atherosclerosis, rheumatoid arthritis, and peritonitis. Malignancy is also an example which has long had a positive association with chronic inflammation [38, 39]. Other conditions are in the process of being explored and the role of inflammation and its etiologic agent are of interest. One such condition is benign prostatic hyperplasia which is known to have an inflammatory etiology [40].

1.3 DISEASES OF THE PROSTATE

Potential associations between HHV-8 and diseases of the prostate, both malignant and benign, are continually being explored. This section serves to outline two conditions, prostate cancer and benign prostatic hyperplasia, and discusses underlying role of inflammation and localization of disease.

1.3.1 Benign Prostatic Hyperplasia

Benign prostatic hyperplasia is one of the most common abnormalities of the prostate. The disease is characterized by an increase in the cell count leading to overgrowth of two types of cells, epithelial and stromal cells [41]. These cells are located in the transition zone and periurethral area, which are located in the central part of the organ [42]. This ultimately constricts the urethra and leads to symptomology known in the medical field as lower urinary tract symptoms (LUTS) [41-44] (Figure 2).

1.3.1.1 Signs and Symptoms

Some men with BPH will remain asymptomatic and not develop LUTS. For those who experience LUTS, these are symptoms either classified as voiding symptoms, also known as obstructive symptoms, including urinary hesitation, reduced urinary stream, and straining while voiding. The second classification of symptoms involves increased urinary retention and storage, also known as irritative symptomology. These symptoms involves, increased urination frequency, increased urgency, and pain and/or burning during urination It is important to note that there are other medical conditions which cause LUTS. One such condition is overactive bladder and conditions such as this must be ruled out when symptoms consistent with BPH are observed [45].

1.3.1.2 Diagnosis

Diagnosis of BPH involves a number of criteria. The first is an abnormal digital rectal examination. Blood tests measuring the prostate specific antigen (PSA) levels are also used. PSA is a serine protease produced in the prostate epithelium. A plasma level greater than 4.0 ng/ml is

used as the cutoff, indicating abnormalities. Ultrasound is also performed to rule out malignancies such as prostate cancer. A number of other tests exist for diagnosis of BPH. These include urinalysis to rule out infections and presence of blood. A test of the overall flow of urine, uroflowmetry, detects reduced flow of urine consistent with BPH. Urethrocystoscopy is a technique used to examine the lower urinary tract is also used. Measuring post void residual urine volume (PVR) after urination is another important diagnostic tool [46].

1.3.1.3 Risk Factors

Several risk factors have been proposed as potential contributors towards BPH. Age is a significant risk factor. BPH affects many men over the age of 50 years. The prevalence of the disease increases with age with an alarming 90% prevalence by 70 years of age. [45, 48] While prevalence is high, not all affected individuals experience symptoms. The next risk factor is family history. If a family member has the disease, it makes it more likely that the individual will develop the condition. Risk factors associated with heart disease also increase the likelihood of developing BPH. These include overweight/obesity, high blood pressure, low High Density Lipoprotein (HDL) cholesterol, diabetes, and lack of physical activity [48].

1.3.1.4 Inflammation

One of the most important risk factors in development of BPH is chronic unregulated chronic inflammation. The triggering event and/or factor for the BPH inflammation is not entirely clear, however infection is one possible factor. In previous studies, BPH specimens, have yielded presence of bacterial and viral strains. In addition, proinflammatory cytokines and

chemokines have been found in stromal cells [45]. A number of cytokines and growth factors including IL-12, and IL-23 were found to be secreted by stromal cells [45, 49]. The secretion of IL-17 as leads to increased downstream production of the cytokines IL-6 and IL-8 [45, 50]. Markers of inflammation such as the C-polysaccharide reactive protein (CRP), were also found to be upregulated [51]. Other factors such as Prostaglandin-endoperoxide synthase 2 (COX)-2 have also been found to be upregulated [45, 52].

1.3.1.5 Treatment

A variety of treatment options are available. These vary from watchful waiting to surgical therapies. The choice for the patient depends on each individual circumstance [47, 53]. Watchful waiting involves monitoring the condition and consulting with a medical professional for effective management of the disease. Medical therapies are also available. These include Alpha-Blockers, 5-alpha-reductase inhibitors (5-ARIs), phosphodiesterase-5 enzyme inhibitors as well combination therapies of the aforementioned medications [54]. Minimally invasive procedures, including transurethral needle ablation (TUNA), transurethral microwave thermotheraphy (TMUT) are other options. Lastly, a variety of surgical therapies are available including prostatectomy, transurethral holmium laser abalation (HoLAP), Transurethral holmium laser enucleation (HoLEP), Holmium laser resection (HoLRP), Photoselective vaporization (PVP), Transurethral incision (TUIP), Transurethral vaporation (TUVP), and Transurethral resection (TURP) [53].

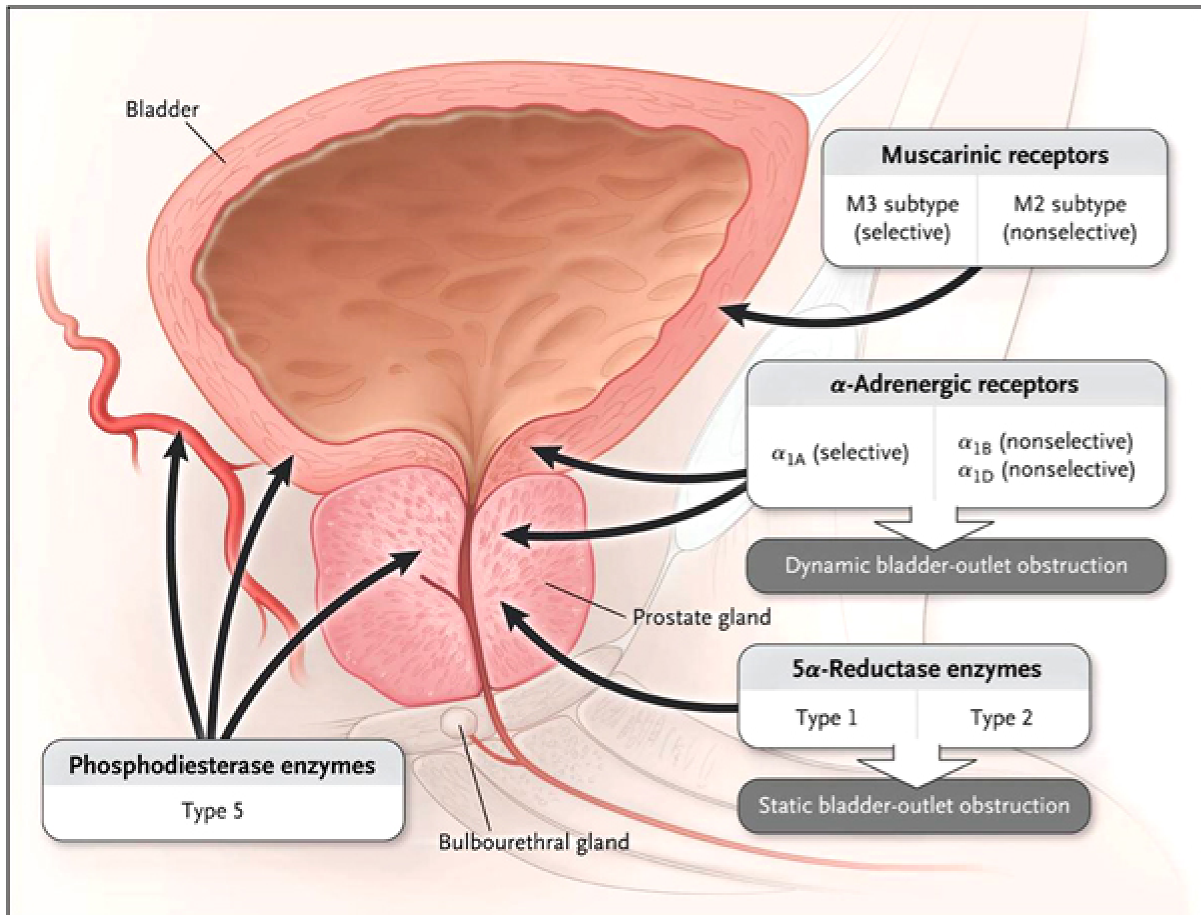


Figure reprinted with permission from *New England Journal Medicine*, *Sarma et al,*. The prostate gland is shown with benign prostatic hyperplasia causing constriction of the urethra leading to lower urinary tract symptoms (LUTS). The localized targets of medical therapies are shown.

Figure 2. Prostate gland with BPH disease

1.3.1.6 Epidemiology of BPH and LUTS

As previously mentioned, age is a risk factor. BPH prevalence is low at 8% of men aged 31-40 years and prevalence increases to 90% by 70 years of age [48]. Not all BPH causes LUTS, which requires treatment. It is estimated that prevalence of LUTS is approximately 50% by age of 70 years which is lower than the prevalence of BPH. The odds of developing moderate to

severe LUTS, increases with an odds ratio of 1.9 by age of 50 years to an odds ratio of 3.4 by 70 years of age [48].

1.3.2 Prostate Cancer

Prostate cancer is a significant malignant disease with high incidence. It is the most frequently diagnosed cancer in men in the US. It is estimated that in 2014, there will be an incidence of 233,000 new cases, with an estimated 29,480 deaths. In men, prostate cancer is the second leading cause of all cancer deaths [55]. 1 in 7 men will be diagnosed with prostate cancer, and of those men, 1 in 36 will die from the disease. The disease involves malignant neoplasm which originates in the outer peripheral zone of the prostate gland [57].

1.3.2.1 Signs and Symptoms

In the early stages, prostate cancer often presents with no symptoms. It is not until more advanced stages of cancer, that may present with symptoms and these may be confused with other disorders such as BPH, and these include problems with voiding, such as interrupted stream, weak stream, and in the worst scenario, the overall inability to urinate [58].

1.3.2.2 Diagnosis

Diagnosis of prostate cancer is done through an evaluation of the medical history and a physical exam. Many of the same techniques used in diagnosis of BPH is used in prostate cancer. In the physical exam, a digital rectal examination is performed. Rectal ultrasound is also used to image the prostate. In addition the prostate-specific antigen is also performed. The prostate biopsy is one of the most important techniques to determine cancer if the PSA and/or the DRE

suggest that cancer might be present [58]. Histopathology is used to determine the presence of cancerous cells in the biopsy. Unfortunately, although biopsies are one of the important techniques in diagnosis, they can yield false negatives, and repeat biopsy procedures may be required. This is due in part to the fact that the biopsy needle used may not capture the cancerous tissue in the prostate [59, 60].

1.3.2.3 Risk Factors

There are risk factors which have been positively associated with development of prostate cancer. These include family history of prostate cancer, advanced age, and ethnicity. In terms of ethnicity, prostate cancer is more prevalent in African-Americans as compared to Caucasians and Asians. This inequity in increased risk holds true regardless of geographical location [57]. In the islands of Trinidad and Tobago, the prevalence of prostate cancer is 3-fold higher in African men compared to Asian Indian descent [61-62]. Genetic factors have been identified which increase risk of prostate cancer development. These include polymorphisms within the androgen receptor gene yielding greater androgenic stimulation. The androgens testosterone and dihydrotestosterone have been shown to increase prostate cancer cell growth [63].

1.3.2.4 Inflammation

An association between prostatic inflammation and development of prostate cancer has been reported. Epidemiologic studies have demonstrated an increased risk in prostate cancer with inflammation with infective etiology. Specifically, infectious agents have been implicated with development of prostatitis (prostate inflammation). It is estimated that as of 2008, approximately 15% of all prostate cancers can be attributed to infectious agents [63, 64]. While a number

viruses have been investigated including herpes simplex (HSV), cytomegalovirus (CMV), and papillomaviruses, there is no evidence for significant increase in risk. Nevertheless, it is believed that infections contribute to a chronic inflammation which increases risk [65]. Infectious agents contributing to inflammation are thought to have a synergistic interaction with other sources of inflammation including diet, environmental exposures, and changes in hormonal production, retrograde urinary reflex and trauma (Figure 3) [54, 57]. Recently, the cytokine IL-6, has been given attention for its role in the inflammatory process. IL-6 is involved in cell proliferation, apoptosis, differentiation, and angiogenesis. Specifically, IL-6 has been shown to promote prostate cancer cell proliferation as well as inhibit apoptosis IL-6 acts as a growth factor in an autocrine and paracrine fashion. Studies have demonstrated that elevated IL-6, is correlated with poorer outcomes. Additionally, the role of the single nucleotide polymorphisms for IL-6 cytokine receptor have been explored with preliminary data demonstrating increased risk, via overproduction of cytokine or increased sensitivity to cytokine [67-69].

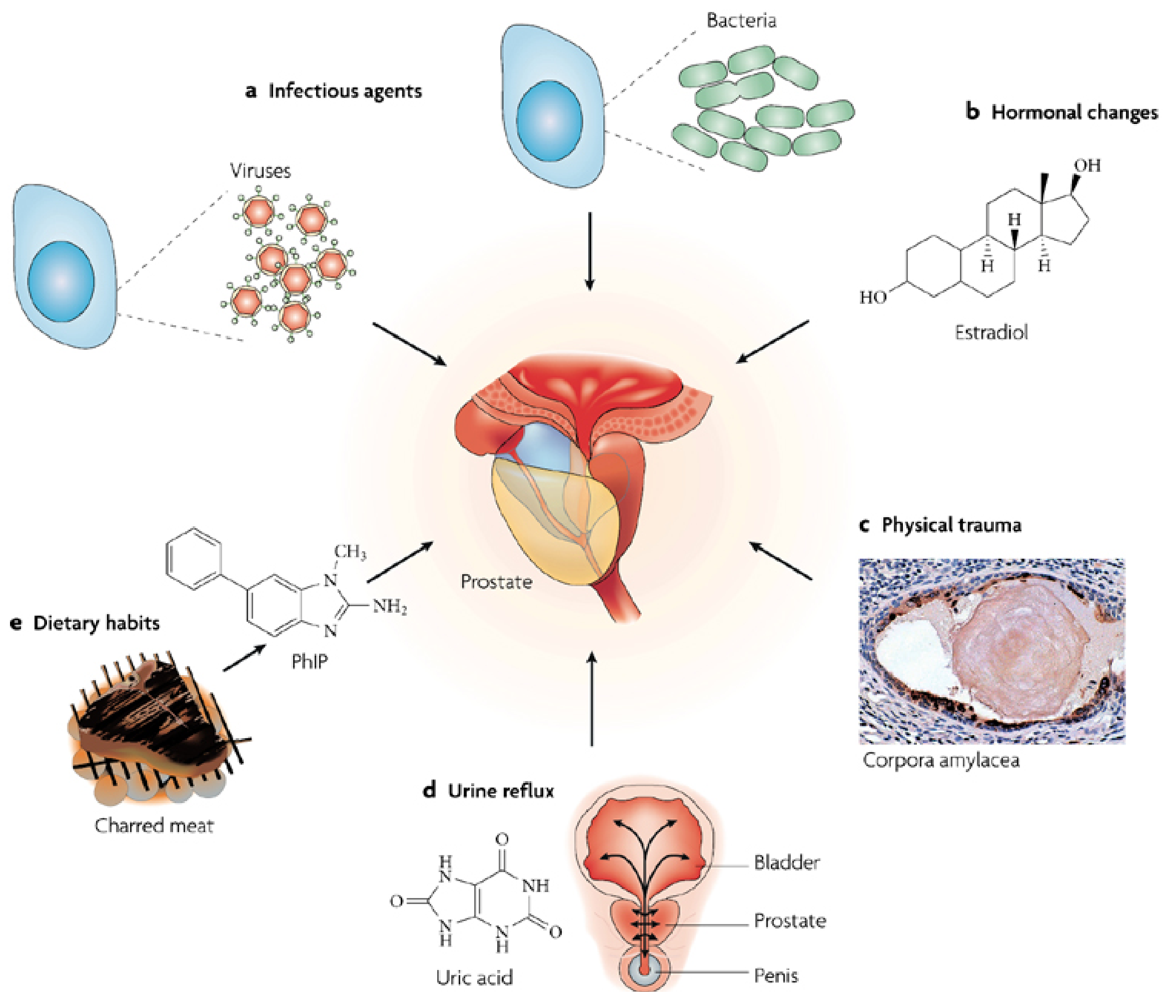


Figure reprinted with permission from *Nature Reviews Cancer*, *De Marzo et al.,* Prostate inflammation can originate from a variety of sources including bacterial and viral infections, changes in hormones, chemical exposure from the diet, and retrograde urine reflux exposing the urogenital system to uric acid.

Figure 3. Sources of prostatic inflammation

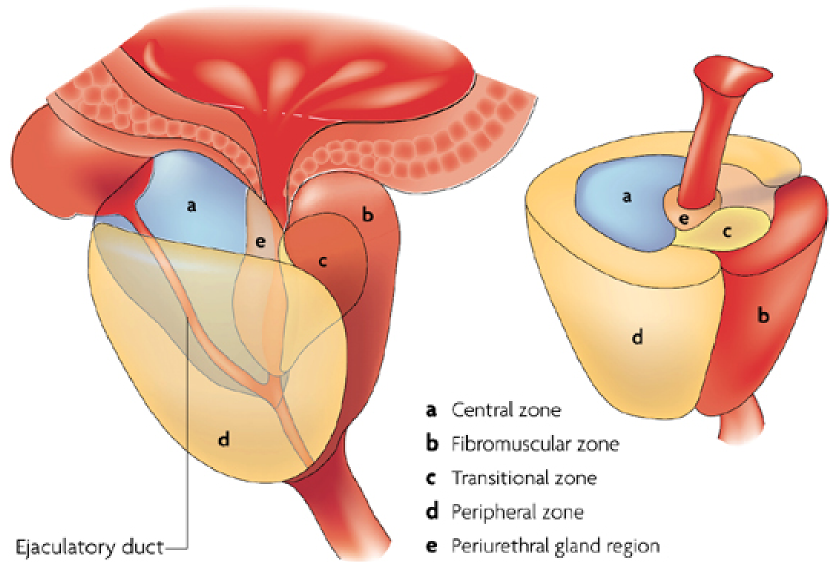
1.3.2.5 Previous Studies Involving HHV-8 Seroprevalence

As previously mentioned, HHV-8 can be transmitted sexually. Considering its oncogenic potential, our lab has published data from a case-control study consisting of a cohort of participants from the Caribbean island nation of Tobago. The cohort included 138 men from Tobago with biopsy confirmed prostate cancer which were designated cases. One hundred forty age matched controls from Tobago with no prostate cancer were designated as controls. Controls in order to be included had to have normal digital rectal exam, and PSA values less than 4.0ng/ml. In addition there were 174 men from Trinidad with no diagnostic evidence of prostate cancer. The distribution of each control group varied. Tobagonians were 97% African, and Trinidadians were 90% Asian Indian descent, and 10% African [70].

Men with prostate cancer were more likely to be HHV-8 seropositive than their age–matched cancer free controls (OR 2.24; 95 C.I. 1.29-3.90) [70].

1.4 RELATIONSHIP BETWEEN BPH AND PROSTATE CANCER

The potential relationship between BPH and an increased risk of development of prostate cancer is highly controversial and continually debated. This is in part due to the fact the diseases share many traits including similar hormone-dependent growth, and an underlying inflammatory component. Currently, general consensus is that BPH is not a precursory condition to prostate cancer. Rather, these two conditions are believed to coexist. This is supported by histology and localization of the disease. As previously mentioned BPH are characterized by nodules with hyperplasia of epithelial and stromal cells in the centrally located transition zone. In contrast to prostate cancer which is an adenocarcinoma which primary develops in epithelial cells in the peripheral zone (Figure 4) [56]. Studies have found that other risk factors including age and family history are strongly predictive of prostate cancer [57]. One recent large scale Danish epidemiological study found that there was an increased risk of prostate cancer incidence and mortality. The authors of this study concluded that the data does not provide a causal link between BPH and Prostate cancer and Scandinavian countries have higher incidence of prostate cancer [72]. One theory is that BPH induces a surveillance bias for prostate cancer [71]. While this is the case, a more recent study has also demonstrated an increased risk of aggressive prostate cancer development in patients with inflammatory BPH. This study was controlled for surveillance bias through analysis of cases and controls in patients with low PSA and patients with no indications for prostate biopsy [73].



	Prostate zone		
	Peripheral	Transition	Central
Focal atrophy			
Acute inflammation			
Chronic inflammation			
Benign prostatic hyperplasia			
High-grade PIN			
Carcinoma			

High prevalence
Medium-high prevalence
Low prevalence
None

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Figure reprinted with permission from *Nature Reviews Cancer*, *De Marzo et al.,* Most prostate cancer adenocarcinomas occur in the peripheral zone, with prevalence decreasing into the transition zone and no prevalence in the central zone. BPH disease occurs with high prevalence in the transition zone decreasing into lower prevalence in the central zone.

Figure 4. Prostate localization and disease occurrence

1.5 CYTOKINES

Cytokines are proteins which serve as signaling mediators altering behavior of other cells. Cytokines act in an autocrine, paracrine, or and/or endocrine fashion binding to receptors on the cell surface leading to signal transduction. The response to the cytokine signal varies and includes processes such as cell differentiation, apoptosis regulation, cytolytic activity, and chemotaxis.. A few examples which are relevant to this thesis is as follows [76]:

IFN-γ – Interferon gamma is a pro-inflammatory cytokine produced primarily by Th1 helper cells, and to a lesser extent, by natural killer cells. The target of this cytokine include monocytes, endothelial cells, and macrophages. Effects include activation, increase in natural killer cell activity, increased expression of class I and class II MHC, B and T cell differentiation as well as macrophage and granulocyte activation.

IL-1- Interleukin 1 is a pro-inflammatory cytokine secreted by the activated macrophages, monocytes, epithelial cells, endothelial cells, and astrocytes. Primary targets, include T and B cells, endothelial cells, hypothalamus and liver. The effects include T-cell activation.

IL-2- Interleukin 2 is a pro-inflammatory cytokine produced by Th cells and serves as a growth factor for T and B cells. It also activates NK cells and monocytes.

IL-4- Interleukin 4 is a pro-inflammatory cytokine produced by macrophages and Th2 cells. The target cells include naïve Th cells allowing for differentiation into Th2 cells, ultimately important for humoral response.

IL-6- Interleukin 6 is a pro-inflammatory cytokine produced by T cells, Macrophages, and Fibroblasts. Its target cells include T and B cells, and liver cells, IL-6 serves as a co-stimulatory molecule, and growth factor. IL-6 is implicated in prostate cancer as it has been shown to be involved in cell proliferation, apoptosis, differentiation, and angiogenesis.

IL-8- Interleukin 8 is a pro-inflammatory cytokine produced by macrophages, epithelial cells, platelets. Its target cells include neutrophils. Its primary effects are activation and chemotaxis.

IL-10- Interleukin 10 is a pro-inflammatory cytokine produced by Th2 cells. Its primary targets include Macrophages and T-cells. The effects include inhibition of antigen presenting cell activity and inhibition of cytokine production.

IL-12- Interleukin 12 is a pro-inflammatory cytokine produced by activated macrophages and dendritic cells. Its primary target includes Naïve T cells allowing differentiation into Th1 helper cells ultimately signaling a cellular immunity response. It also aids in IFN-γ production.

TNFα- is produced by activated macrophages. This cytokine is involved in recruitment of neutrophils and macrophages to appropriate sites of infection. As with IL-1, TNF is involved with fever responses.

1.6 HYPOTHESIS

As previously described in the Tobago prostate cancer case-control study, data have indicated a positive association between HHV-8 and increased risk of development of prostate cancer (OR=2.24). Further, it has previously been demonstrated in our lab, that there is increased macrophage infiltrate in HHV-8 seropositive men. Given that HHV-8 is an infectious agent and sexually transmitted infection, it is hypothesized that HHV-8 establishes a chronic infection in the prostate leading to chronic inflammation. Since BPH is a disease associated with inflammation, it is hypothesized that HHV-8 infection contributes to BPH. Further, it is hypothesized that serum levels of proinflammatory cytokines will be elevated in men with BPH compared to controls.

1.7 SPECIFIC AIMS

The present study is based on a previous published study conducted by a member of our lab, Dr. Linda Hoffman utilizing serum samples from a cohort of men the island of Tobago collected by Dr. Clareann Bunker Ph.D, in the Department of Epidemiology, University of Pittsburgh. This study concluded that HHV-8 seropositivity is significantly elevated in men with prostate cancer when compared with control subjects. This suggests that HHV-8 may have a role in the development of prostate cancer [70].

One hundred sixty eight serum samples of the Tobago cohort who were diagnosed with BPH were obtained from the laboratory of Dr. Clareann Bunker Ph.D. Inclusionary criteria included normal baseline biopsy, no prostate cancer diagnosis, and PSA ≥4.0 ng/ml. Patients were 40-79 years of age.

Serum samples of patients with a negative diagnosis of prostate cancer (controls) previously tested for HHV-8 serology by Hoffman et al. from the Tobago cohort (n=234) were also utilized.

1.7.1 Specific Aim 1

Test the hypothesis that a positive association exists between HHV-8 seroprevalence and BPH development by comparing seroprevalence among men aged 40-79 years diagnosed with BPH to controls. BPH subjects were defined by PSA ≥4.0 ng/ml, and a normal baseline biopsy. The age-matched controls were defined by PSA values of <4.0ng/ml and normal digital rectal exam. The HHV-8 serology on the control group was previously determined and reported by Hoffman et al. [70].

Aim 1 is accomplished utilizing an HHV-8 enhanced lytic immunofluorescent assay that detects the presence of antibodies to lytic HHV-8 antigens. Screening for seropositivity was performed on 168 serum samples from men with BPH. Serology data was compared with previously obtained age-matched controls.

1.7.2 Specific Aim 2

The specific objectives of AIM 2 were to test the hypothesis that proinflammatory cytokine serum levels are elevated among men who are diagnosed with BPH. Further, a second objective of this study is to test the hypothesis that HHV-8 causes an increase in the proinflammatory cytokines. Comparisons between HHV-8 seropositive men and seronegative men in both BPH and control groups will be made. Final comparisons between BPH and control diagnosis in seropositive and seronegative groups will be made.

Aim 2 is accomplished through the testing of serum samples of one hundred twenty BPH patients; 63 HHV-8 seropositive and 57 HHV-8 seronegative. An age matched control group

consisted of 39 HHV-8 seropositive and 48 HHV-8 seronegative samples from men with normal PSA and DRE.

2.0 MATERIALS AND METHODS

2.1 CELL CULTURE

2.1.1 BCBL-1

The BCBL-1 cell line was developed from a body cavity based lymphoma cell line infected with HHV-8 but not EBV. These cells can be induced to produce HHV-8 with the phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA). Cells are cultured in RPMI 1640 with L-glutamine (Corning CellGro # 10-040-CV), 10% heat inactivated Fetal Bovine Serum (Gemini #100-506), and 1x Gentamicin (Lonza # 17-518Z).

2.2 IMMUNOFLUORESCENCE ASSAY

2.2.1 Slide Preparation

To determine presence of HHV-8 lytic antigens, immunofluorescence assay was performed as previously described [70]. 5mm 12-well slides (Fisher Scientific #12-580-23) were coated with 10% poly-l-lysine and allowed to air dry. One liter of BCBL-1 in a T175 flask was set up at 2.5×10^5 cells/ml overnight at 37°C in the incubator. The following day, cells were induced with TPA at a final concentration of 20ng/ml. Cells were allowed to incubate for an additional five days. Following incubation, cells were harvested. Briefly, cells were transferred

to 250ml bottles and spun at 3500rpm at room temperature for 25 minutes. Cells were washed with 1X PBS three times, reducing volume of PBS wash each time. Cells were resuspended in one-tenth of final volume of PBS wash in 4% buffered paraformaldehyde and incubated for ten minutes at room temperature to fix cells. Cells were pelleted and washed twice to remove paraformaldehyde. Following incubation, cells were permeabilized in 0.05% Triton-X 100 for ten minutes at room temperature. Cell counts were performed using a hemocytometer and cells were resuspended in 1X PBS to yield a concentration between 1.5-2.5x10^7 cells/ml. 10µl Aliquot of cell suspension were added to each slide well. Slides were allowed to air dry and frozen at -20°C until assay was performed.

2.2.2 Enhanced Lytic Immunofluorescent Assay

HHV-8 seroprevalence of serum samples were examined using enhanced lytic immunofluorescent assay. Slides were removed from -20°C freezer, and place in a humidified chamber for 10 minutes. Following humidification, slides were blocked with 20µl per well of 10% goat serum (Gibco# 16210) at 37°C for 1 hour. Two dilutions of serum samples (Primary), at 1:50, and 1:100 were prepared during incubation period. Following blocking incubation period, goat serum was aspirated, and 20µl of each primary sample dilution was added to separate wells of individual slide. Primary was allowed to incubate for 1 hour at 37°C. Following primary incubation, wells were aspirated and washed in 1x PBS. Secondary monoclonal mouse Anti-Human IgG (Fc Specific) Clone CG-7 antibody (Sigma#I-6260) was diluted 1:200 in 10% serum and 20ul was added to each well of the slide. Slides were incubated for 37°C for 1 hour. Following secondary incubation, wells were aspirated and slides were washed in 1xPBS. Tertiary anti-mouse IgG gamma-fluorescein antibody (Roche #100813) was diluted 1:100 in 10% goat

serum and added to each well under light sensitive conditions. Slides were incubated for 1 hour at 37°C. Post incubation, tertiary antibody was aspirated and slides were washed in 1x PBS in light sensitive conditions. A final overnight incubation step was performed in PBS. Slides were analyzed in a blinded manner with a fluorescence light microscope utilizing a 1:50 cutoff value.

2.3 MESO SCALE DISCOVERY SYSTEM

The Meso Scale Discovery MSD® immunoassay platform was used to measure the levels of pro-inflammatory cytokines in prostate serum samples. The assay is a sandwich immunoassay utilizing electrochemiluminescence detection technology. Electrodes within the prepared assay plate are attached to capture antibodies specific for the analytes IFN-γ, IL-1β, IL-4, IL-6, IL-8, IL-10, IL12p70, (MSD, Proinflammatory Panel 1- K15049D). Serum samples were diluted utilizing manufacturer's supplied diluent at a 2-fold dilution, added in duplicate to the supplied 96 well plate, and incubated at room temperature shaking for two hours. Plates were washed three times with manufacturer supplied wash buffer and 25µl of manufacturer supplied reconstituted detection antibody was added to each well. Plate was incubated at room temperature shaking for two hours. Following detection, antibody incubation plates were washed three times with wash buffer. 150µl of manufacturer supplied read buffer was added to each well. Plates were read on a MSD™ SECTOR imager 2400. Analysis of data was performed in MSD™ Discovery Software Suite. Cytokine levels that were below the standard curve were given the value of 0, and values above the standard curve were given the highest concentration in the standard curve.

2.4 STATISTICAL ANALYSIS

2.4.1 AIM 1

Statistical analysis was performed in the serology study utilizing a two-way contingency table to calculate chi-squared value with Fisher's exact test with significance level $p<0.05$, as appropriate.

2.4.2 AIM 2

Statistical analysis was performed in the pro-inflammatory cytokine study utilizing an Independent samples Mann-Whitney U test with significance level at $p<0.05$ as appropriate.

3.0 RESULTS

3.1 AIM 1

AIM 1 was performed to test the hypothesis that a positive association exists between HHV-8 seroprevalence and BPH, an inflammatory prostate condition. Among the 168 Tobago men with BPH, HHV-8 seroprevalence was found to be 42.9% which was significantly higher than the seroprevalence among 140 age-matched cancer-free controls obtained from the Tobago HHV-8 prostate cancer study by Hoffman et al. (22.9%; p=0.000, odds ratio (OR) 2.51, 95% confidence interval (CI) 1.48-4.26) (Table 1). A second comparison between HHV-8 serology in prostate cancer cases and BPH cases was made to test for differences between these two inflammatory mediated conditions. In the 138 Tobago men with biopsy-confirmed cancer cases, HHV-8 seroprevalence was found to be 39.9% which was not significantly different compared to BPH cases (42.9% p=0.64, OR=1.12, 95% CI 0.69-1.82). Previous studies have only compared prostate cancer and controls, therefore comparison between prostate cancer to any other status, including BPH and controls was of interest. Combined BPH and cancer-free control group HHV-8 seroprevalence was found to be 33.7%. This was not significantly less compared to biopsy-confirmed prostate cancer group (39.9%, p=0.239, OR= 0.77, 95% CI 0.50-1.18). There was an interest in comparing the serology of any abnormal prostate condition (cancer and BPH) to age matched controls. HHV-8 seroprevalence was found to be 41.5% in the aggregate group which

was significantly greater than age-matched controls (22.9%, p=0.000, OR=2.38, 95% CI 1.48-3.86).

Table 1. HHV-8 Serology of Men in the Island Nation of Tobago

HHV-8 Serology Group Comparisons	p-value	OR(95% CI)
Tobago BPH (72/168; 42.9%) vs. Age Matched Controls (32/140; 22.9%)	**0.000**	2.51(1.48-4.29)
Tobago BPH (72/168; 42.9%) vs. Prostate Cancer Cases (55/138; 39.9%)	0.643	1.12(0.69-1.82)
Tobago Prostate Cancer Cases (55/138;39.9%)* Tobago Age-matched Controls (32/140; 22.9%)*	**0.003**	2.24(1.29-3.90)
Tobago Prostate Cancer Cases and Tobago BPH (127/306; 41.5%) vs. Tobago Age-matched Controls (32/140; 22.9%)	**0.000**	2.38(1.48-3.86)
Tobago BPH Cases and Age-matched Controls (104/209;33.7%) vs. Tobago Prostate Cancer (55/138;39.9%)	0.239	0.77(0.50-1.18)
*Data derived from Tobago HHV-8 prostate cancer study, Hoffman et al [70].		

Statistical analysis was performed utilizing Chi-squared with Fisher's exact t-test at significance at p=0.05

3.2 AIM 2

Aim 2 was performed to test the hypothesis that proinflammatory cytokine serum levels are higher among men who are diagnosed with BPH as well as men who are HHV-8 seropositive. Serum samples of one hundred twenty BPH patients; 63 HHV-8 seropositive and 57 HHV-8 seronegative were used. An age matched control group was established utilizing previously obtained and tested samples from the Tobago cohort study selecting 39 HHV-8 seropositive and 48 HHV-8 seronegative samples.

The mean levels of each pro-inflammatory cytokine are listed (Table 2). Notably, IL-8 was found to be present in greater mean levels compared to other cytokines in all groups.

Table 2. Serum Cytokine Levels Stratified by Seropositivity and Case Status

Cytokine	Control, HHV-8 Seropositive (N=39) (pg/ml) Mean±SD	Controls, HHV-8 Seronegative (N=48) (pg/ml) Mean±SD	BPH, HHV-8 Seropositive (N=63) (pg/ml) Mean±SD	BPH, HHV-8 Seronegative (N=57) (pg/ml) Mean±SD
IFN-γ	2.22± 2.58	2.28± 2.43	6.99±11.89	5.22± 6.51
IL-10	0.21± 0.32	0.29± 1.04	0.45± 0.55	0.42± 0.70
IL-13	0.57± 0.43	0.32± 0.35	0.46± 0.53	0.44± 0.35
IL-12p70	6.75± 6.52	1.92± 2.21	4.89± 5.35	5.31± 4.74
IL-1β	1.23± 6.68	0.17± 0.52	1.45± 6.38	0.15± 0.27
IL-2	0.17± 0.25	0.36± 0.55	1.57± 6.11	0.35± 0.55
IL-4	0.08± 0.11	0.25± 0.54	0.37± 0.29	0.20± 0.19
IL-6	21.08± 127.57	1.17± 2.57	8.92± 52.20	1.55± 1.61
IL-8	251.21± 1392.15	33.04± 84.86	345.84± 820.70	223.53± 564.84
TNFα	0.40± 0.84	0.77± 2.51	1.92±1.83	1.60± 1.53

An initial comparison was made between controls including all serology and men diagnosed with BPH (Table 3).

Table 3. Comparison of Pro-Inflammatory Cytokines between Control and BPH cases

Cytokine	Control (N=87) (pg/ml) Mean±SD	BPH (N=120) (pg/ml) Mean±SD	p-value
IFN-γ	2.22± 2.48	6.15± 9.71	**0.000**
IL-10	0.26± 0.80	0.44± 0.62	**0.000**
IL-13	0.43± 0.41	0.45± 0.45	0.915
IL-12p70	4.08± 5.23	5.09± 5.05	0.163
IL-1β	0.65± 4.49	0.83± 4.65	**0.024**
IL-2	0.27± 0.45	0.99± 4.46	**0.001**
IL-4	0.17± 0.42	0.28± 0.26	**0.000**
IL-6	10.1± 85.40	5.42± 37.87	**0.000**
IL-8	130.84± 933.92	287.74± 710.51	**0.000**
TNFα	0.60± 1.95	1.77± 1.70	**0.000**

Statistical analysis was performed utilizing an independent samples Mann-Whitney U-test with significance at $p<0.05$.

Analyses of the levels of pro-inflammatory cytokines revealed seven out of ten were significantly elevated in BPH patients compared to controls ($p<0.05$). One exception was IL-6 which was significantly higher among the control group. Two of the cytokines, IL-13 and IL-12p70 were not significantly different.

BPH cases and controls groups were individually stratified on the basis of HHV-8 serology and comparisons were made to determine the effect of HHV-8 infection on pro-inflammatory cytokine levels. Among the control group, the cytokines IL-13, IL-12p70 and IL 1β were significantly higher among the HHV-8 seropositive subjects ($p<0.05$). There were no

significant differences between groups with IFN-γ, IL-6, IL-8 and IL-10 cytokines and TNFα (Table 4).

Table 4. Comparison of Pro-Inflammatory Cytokines in Control Group Stratified by HHV-8 Serology

Cytokine	**Controls, HHV-8 Seropositive (N=39) (pg/ml) Mean +S.D.**	**Controls, HHV-8 Seronegative (N=48) (pg/ml) Mean +S.D.**	**p-value**
IFN-γ	2.22± 2.58	2.28± 2.43	0.626
IL-10	0.21± 0.32	0.29± 1.04	0.590
IL-13	0.57± 0.43	0.32± 0.35	**0.001**
IL-12p70	6.75± 6.52	1.92± 2.21	**0.000**
IL-1β	1.23± 6.68	0.17± 0.52	**0.000**
IL-2	0.17± 0.25	0.36± 0.55	**0.041**
IL-4	0.08± 0.11	0.25± 0.54	**0.000**
IL-6	21.08± 127.57	1.17± 2.57	0.207
IL-8	251.21± 1392.15	33.04± 84.86	0.778
TNFα	0.40± 0.84	0.77± 2.51	0.481

Statistical analysis was performed utilizing an independent samples Mann-Whitney U-test with significance at $p<0.05$.

Stratified HHV-8 serology comparison of pro-inflammatory cytokine levels among BPH cases showed significantly higher levels of IL-1β, IL-2, and IL-4 among seropositive subjects. There were no significant differences in the remaining cytokines between BPH HHV-8 seropositive and seronegative groups (Table 5).

Table 5. Comparison of Pro-Inflammatory Cytokines in BPH group Stratified by HHV-8 Serology

Cytokine	BPH, HHV-8 Seropositive (N=63) (pg/ml) Mean +S.D.	BPH, HHV-8 Seronegative (N=57) (pg/ml) Mean +S.D.	p-value
IFN-γ	6.99±11.89	5.22± 6.51	0.815
IL-10	0.45± 0.55	0.42± 0.70	0.556
IL-13	0.46± 0.53	0.44± 0.35	0.447
IL-12p70	4.89± 5.35	5.31± 4.74	0.327
IL-1β	1.45± 6.38	0.15± 0.27	**0.001**
IL-2	1.57± 6.11	0.35± 0.55	**0.001**
IL-4	0.37± 0.29	0.20± 0.19	**0.000**
IL-6	8.92± 52.20	1.55± 1.61	0.688
IL-8	345.84± 820.70	223.53± 564.84	0.791
TNFα	1.92±1.83	1.60± 1.53	0.155

Statistical analysis was performed utilizing an independent samples Mann-Whitney U-test with significance at $p<0.05$.

Comparison of circulating pro-inflammatory cytokine levels among BPH cases and controls who are HHV-8 seropositive demonstrated significant increases in IFN-γ, IL-10, IL-2, IL-4, IL-8 and TNFα in the BPH cases while IL-13 and IL-6 were significantly elevated in the controls. The levels of IL-12p70 and IL-1β were not significantly different between these HHV-8 seropositive groups (Table 6).

Table 6. Comparison of Pro-Inflammatory Cytokines in HHV-8 Seropositive Groups

Cytokine	BPH, HHV-8 Seropositive (N=63) (pg/ml) Mean +S.D.	Control, HHV-8 Seropositive (N=87) (pg/ml) Mean +S.D.	p-value
IFN-γ	6.99±11.89	2.22± 2.58	**0.000**
IL-10	0.45± 0.55	0.21± 0.32	**0.000**
IL-13	0.46± 0.53	0.57± 0.43	**0.049**
IL-12p70	4.89± 5.35	6.75± 6.52	0.055
IL-1β	1.45± 6.38	1.23± 6.68	0.521
IL-2	1.57± 6.11	0.17± 0.25	**0.000**
IL-4	0.37± 0.29	0.08± 0.11	**0.000**
IL-6	8.92± 52.20	21.08± 127.57	**0.000**
IL-8	345.84± 820.70	251.21± 1392.15	**0.000**
TNFα	1.92±1.83	0.40± 0.84	**0.000**

Statistical analysis was performed utilizing an independent samples Mann-Whitney U-test with significance at $p<0.05$.

Comparison of circulating pro-inflammatory cytokine levels among BPH cases and controls who are HHV-8 seronegative demonstrated significant increases in IFN-γ, IL-10, IL-12p70, IL-6, IL-8 and TNFα in the BPH cases while IL-1β and IL-4 were significantly different between these HHV-8 seronegative groups (Table 7).

Table 7. Comparison of Pro-Inflammatory Cytokines in HHV-8 Seronegative Groups

Cytokine	BPH, HHV-8 Seronegative (N=57) (pg/ml) Mean +S.D.	Control, HHV-8 Seronegative (N=87) (pg/ml) Mean +S.D.	p-value
IFN-γ	5.22± 6.51	2.28± 2.43	**0.000**
IL-10	0.42± 0.70	0.29± 1.04	**0.000**
IL-13	0.44± 0.35	0.32± 0.35	0.059
IL-12p70	5.31± 4.74	1.92± 2.21	**0.000**
IL-1β	0.15± 0.27	0.17± 0.52	**0.000**
IL-2	0.35± 0.55	0.36± 0.55	0.069
IL-4	0.20± 0.19	0.25± 0.54	**0.013**
IL-6	1.55± 1.61	1.17± 2.57	**0.000**
IL-8	223.53± 564.84	33.04± 84.86	**0.000**
TNFα	1.60± 1.53	0.77± 2.51	**0.000**

Statistical analysis was performed utilizing an independent samples Mann-Whitney U-test with significance at $p<0.05$.

4.0 DISCUSSION

BPH is a prostate disease which is believed to have inflammatory etiology. Underlying factors that drive this inflammation include a possible infective origin. This has been demonstrated in studies which have previously found bacterial and viral strains in prostate samples [45, 48]. As such, a pro-inflammatory response to a pathogen may lead to chronic inflammation. Considering HHV-8 is a sexually transmitted infection and has previously been associated with prostate cancer, another disease with an inflammatory etiology, there was interest in exploring a potential association between HHV-8 and BPH. This study is one of the first to explore this potential relationship.

4.1 AIM 1

The objective of AIM 1 was to test the hypothesis that a positive association exists between HHV-8 seroprevalence and BPH development. This was accomplished through a case-control study utilizing serum samples originally collected in the Tobago prostate study [70]. BPH cases were defined as men from Tobago 40-79 years old with PSA ≥4.0 ng/ml and normal three-core baseline biopsy. The control group had PSA values of <4.0ng/ml, and normal digital rectal exam. Considering men of African descent including Trinidad and Tobago are found to have higher prevalence HHV-8 serology and higher prevalence of prostate diseases, including prostate cancer, this population is ideal to study to test for a possible association. Serum samples were

examined for HHV-8 antigen utilizing enhanced lytic immunofluorescent assay. This assay is a long established method of detecting HHV-8 antigens [74].

This study found that HHV-8 seropositivity was significantly higher among men with BPH, compared to age-matched controls with almost a two-fold difference in HHV-8 serology between these two groups. This alone provides preliminary evidence of a positive association between HHV-8 seroprevalence and the development of BPH, however is insufficient. A second comparison was made between BPH and prostate cancer cases. As both of these diseases are of inflammatory etiology, and there is a positive association of HHV-8 and prostate cancer, there was interest in comparing serology in BPH and prostate cancer. Interestingly, there was no significant difference with BPH having approximately 3% higher serology. This provides additional evidence of a possible association. A third comparison was made between prostate cancer and any other prostate status including BPH and normal prostate condition. If an association between BPH and HHV-8 was present, it would be expected that any significant difference between prostate cancer and all other prostate status would no longer exist as serology levels would increase in the aggregate of BPH and age-matched control group. The data demonstrated that there was an 11 % difference in serology between age-matched control alone and the aggregate of Tobago BPH cases and age-matched controls. Additionally, no significant difference was observed between the aggregate group and prostate cancer cases.

Together, these aforementioned results could provide evidence to suggest an association between HHV-8 and BPH, however, other factors must be considered. For instance, it is estimated 10-20% of biopsies are false negatives, raising the possibility that a patient could have prostate cancer instead of BPH, assuming presence of abnormal PSA. Considering our BPH patients did not have abnormal digital rectal exam as inclusionary criteria, a reliance is made on

abnormal PSA and a normal biopsy. Therefore, a patient could have prostate cancer incorrectly diagnosed as BPH, elevating a potential association. Second, considering there has been considerable debate regarding the relationship between BPH and the development of prostate cancer, and it has been found that both these conditions can coexist simultaneously, only limited conclusions can be made about a possible association between BPH and HHV-8. In order to address these concerns, a comparison was performed between all abnormal prostate conditions to age-matched controls. Close to a two-fold increase in HHV-8 serology was found in abnormal prostate, with a significant difference between two groups.

These data suggest a possible association with HHV-8 and BPH development. It is believed HHV-8 sets up an infection in both a lytic and latent state, which allows for the facilitation of chronic inflammation.

4.2 AIM 2

The objective of AIM 2 was to test the hypothesis that proinflammatory cytokine serum levels were higher among men who were diagnosed with BPH compared to control patients without BPH. A second objective was to test the hypothesis that HHV-8 infection causes an increase proinflammatory cytokines. Statistical analysis was performed utilizing an independent samples Mann-Whitney U-test with significance at $p<0.05$.

This was accomplished through the testing of prostate serum samples of one hundred twenty BPH patients; 63 HHV-8 seropositive and 57 HHV-8 seronegative. Serum samples for tested for IFN-γ, IL-10, IL-13, IL-12p70, IL-1β, IL-2, IL-4, IL-6, IL-8, and TNFα. An age matched control group from the Tobago prostate control group selecting 39 HHV-8 positive and

48 HHV-8 negative samples. Non-parametric independent sample Mann-Whitney U statistical tests were used to test for significant differences between two groups.

As BPH is an inflammatory disease, it is expected that there will be an increase in inflammatory cytokines present in serum, regardless of serology status. Therefore, a comparison was made between the mean levels in the control and the BPH group, and significant differences were observed in eight of the ten cytokines including IL-6 and IL-8 (Table 3). This was consistent with other reported data indicating presence of increased IL-6 and IL-8 in BPH patients [45, 50]. To control for other factors including HHV-8 virus infection, this same comparison was made in seronegative individuals (Table 7). Again, a significant elevation in cytokines was observed in BPH diagnosed individuals.

Having observed this phenomenon, control and BPH groups were stratified by HHV-8 serology status (Table 2, 4, and 5). Examining both the HHV-8 serology stratified control and the BPH groups, the level of pro-inflammatory cytokines become significantly elevated in HHV-8 seropositive individuals, compared to seronegative individuals in both groups. Supporting this observation was the significant elevation in mean cytokine levels in seropositive individuals between BPH diagnosed patients and control group (Table 6). This observation is expected considering BPH induces significant increase and virus infection also leads to a significant increase in cytokine release. Therefore, a synergistic effect is expected.

In summary, the data support the conclusion that BPH leads to increased cytokine response consistent with a chronic inflammatory state. HHV-8 infection was also found to contribute to an increased cytokine elevation in BPH cases and control group. This study was the first of its kind to demonstrate a potential association between HHV-8 and BPH, and provided preliminary evidence to support increased pro-inflammatory response. Further studies

are needed to strengthen this association and provide further evidence of an inflammatory cytokine response.

4.3 LIMITATIONS

While this study was informative, there are limitations which need to be addressed. This study utilized samples from the Island of Tobago, which were obtained between the years 1998-2000. The possibility exists that the integrity of these samples may have been compromised due to mishandling of samples after collection leading a loss of overall representativeness of *in vivo* cytokine levels. Limitations also exist with this study design. This study was a cross-sectional study of all serum samples. To get a better understanding of a potential relationship between HHV-8 infection and BPH, a longitudinal study should be performed. HHV-8 is a herpes virus, and as such has the ability to enter latent and lytic stages. A longitudinal approach would allow one to explore associations in each stage. A second issue is the fact that the study did not utilize individually age-matched cases and controls. Rather, each entire group was matched by age. Because prevalence of BPH varies with age, utilizing matching is importance. Further, this study failed to take into account other possible viral infections, including HIV-1, due to restrictions placed by Institutional Review Board. Therefore, limited conclusions on cytokine release and modulation can be made.

5.0 FUTURE DIRECTIONS

Future studies should aim to address limitations from this study in order to strengthen evidence for a potential association between HHV-8 and benign prostatic hyperplasia. This study was this involved an examination of a single time point where sample was collected for each study participant. Future studies should be longitudinal observing potential changes in HHV-8 serology, accompanied pro-inflammatory cytokine release, and prostate health, given that the prevalence of this disease increases with age. This would provide more information and help to confirm an association.

While the serology was determined in this study through enhanced immunofluorescence assay to establish serology, it would be useful to perform end-point antibody titers utilizing this method. From this data, one could examine if a correlation is present between end point titer and cytokine profiles. Additionally, *In vitro* studies in the laboratory measuring pro-inflammatory cytokines after HHV-8 infection in prostate cell lines should be performed.

6.0 PUBLIC HEALTH SIGNIFICANCE

Benign Prostatic Hyperplasia is a disease with a prevalence of up to 80% of men 70-79 years of age, and can reduce quality of life significantly. At the current time, the pathophysiology of BPH is not entirely understood. While current evidence suggests inflammation is involved in overall etiology, factors which contribute to this inflammation need to be elucidated. This study demonstrated a positive association between HHV-8 and development of BPH. By further understanding the way infectious agents contribute to the pathophysiology of this disease and the pro-inflammatory response profile in seropositive patients, one can further understand the inflammatory process involved in BPH. While it can conceded that many factors besides infectious diseases are involved and may have an interaction in increasing risk of development of BPH, this study provides a foundation to gain an understanding about the role of an infectious co-factor. Upon validation of this research, one can use this data to emphasize measures to prevent spread of sexually transmitted infections including HHV-8. Further understanding of other factors and their contributing role in inflammatory pathway including diet, environmental, hormonal, and genetic factors could allow the eventual reduction of disease burden.

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