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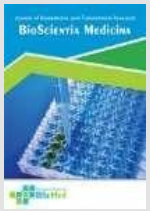

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The Inflammatory Correlates of Hypothalamic-Pituitary-Gonadal Axis Dysfunction in Antiretroviral-Naïve Men with HIV: A Cross-Sectional Analysis of the TNF-α and Testosterone Relationship

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A B S T R A C T

Background: In men with untreated Human Immunodeficiency Virus (HIV), the mechanisms underlying testosterone deficiency remain incompletely defined. Chronic inflammation is hypothesized to be a key factor in disrupting the hypothalamic-pituitary-gonadal (HPG) axis. This study aimed to investigate the association between the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) and the HPG axis and to assess this relationship while accounting for nutritional status in ART-naïve men. **Methods:** We conducted a cross-sectional study of 40 ART-naïve men with HIV in Palembang, Indonesia. We measured serum total testosterone, Luteinizing Hormone (LH), TNF-α, Interleukin-6 (IL-6), and high-sensitivity C-reactive protein (hs-CRP). Hypogonadism was classified as primary (low testosterone, high LH) or secondary (low testosterone, low/normal LH). Spearman's correlation was used to assess bivariate relationships. A multiple linear regression analysis was performed to determine the independent association of TNF-α and Body Mass Index (BMI) with testosterone levels. **Results:** All 40 participants (100%) presented with secondary (hypogonadotropic) hypogonadism, characterized by a median total testosterone of 6.48 pg/mL and an inappropriately normal median LH of 3.86 mIU/mL. Serum TNF-α was significantly elevated (median: 10.32 pg/mL). A moderate negative correlation was found between TNF-α and total testosterone (ρ = -0.411, p = 0.008). In the multivariate regression model, both higher TNF-α levels (β = -0.38, p = 0.011) and lower BMI (β = 0.45, p = 0.003) were significant, independent predictors of lower total testosterone. The model explained 34.6% of the variance in testosterone levels (Adjusted R^2 = 0.346). **Conclusion:** Our findings demonstrate a universal prevalence of secondary hypogonadism in ART-naïve men with HIV. This HPG axis dysfunction is strongly and independently associated with both the magnitude of systemic inflammation, marked by TNF-α, and the severity of malnutrition. These results suggest a complex interplay where inflammation and poor nutritional status act as distinct, synergistic contributors to the profound endocrine disruption seen in untreated HIV infection.

1. Introduction

The global success of highly active antiretroviral therapy (HAART) has profoundly altered the natural history of human immunodeficiency virus (HIV) infection, converting it from an acute, terminal illness into a manageable chronic disease.[1] This triumph of modern medicine has extended lifespans dramatically, but in doing so, has revealed a complex landscape of

non-AIDS-defining comorbidities that contribute to a state of accelerated aging and increased frailty among people living with HIV (PLWH).[2] These comorbidities, including cardiovascular disease, metabolic syndrome, neurocognitive decline, and a spectrum of endocrinopathies, persist as major clinical challenges, underscoring the reality that virological suppression alone does not equate to a full restoration of physiological health.[3]

Central to the pathogenesis of these non-infectious comorbidities is a state of persistent, low-grade chronic inflammation and immune activation.[4] Even with effective viral suppression, the latent HIV reservoir, microbial translocation from a compromised gut barrier, and co-infections contribute to a systemic inflammatory milieu that differs significantly from that of HIV-negative individuals. In the context of untreated, viremic HIV, this inflammatory state is magnified exponentially. Uncontrolled viral replication acts as a potent and continuous stimulus for a massive counter-regulatory immune response, characterized by the systemic release of a cascade of pro-inflammatory cytokines, including Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 (IL-1), and Interleukin-6 (IL-6).[5] This "cytokine storm" is not merely a marker of disease activity but an active participant in HIV pathogenesis, contributing to CD4+ T-cell apoptosis, viral replication, and the clinical wasting syndrome (cachexia) characteristic of advanced AIDS.[6]

Among the most common and clinically debilitating endocrinopathies observed in men living with HIV (MLWH) is hypogonadism, a syndrome of testosterone deficiency. This condition has profound negative impacts on multiple organ systems, adversely affecting sexual function (decreased libido, erectile dysfunction), mood (depression, irritability), body composition (loss of muscle mass, increased adiposity), bone mineral density (osteoporosis), and overall quality of life.[7] In the pre-ART era, the prevalence of hypogonadism was alarmingly high, affecting up to 70% of men with advanced disease. While its prevalence has decreased with the advent of ART, it remains significantly more common in MLWH than in their age-matched, HIV-negative peers, with contemporary estimates ranging from 13-40%.

The pathophysiology of HIV-associated hypogonadism is multifactorial and can be mechanistically categorized.[8] Primary hypogonadism results from direct testicular failure (a gonadal defect), leading to low testosterone and a compensatory increase in pituitary gonadotropins, namely Luteinizing Hormone (LH). In contrast, secondary (or hypogonadotropic) hypogonadism arises from a defect at the level of the hypothalamus or pituitary gland (a central defect), resulting in low testosterone due to insufficient LH secretion. In this central form, LH levels are either low or inappropriately normal in the face of low circulating testosterone, indicating a failure of the central feedback mechanism. A growing body of evidence suggests that secondary hypogonadism is the predominant form in HIV, pointing towards a systemic, rather than a localized testicular, etiology.

This observation has fueled the central hypothesis that the chronic inflammatory state of HIV acts as a primary disruptor of the hypothalamic-pituitary-gonadal (HPG) axis. Pre-clinical and clinical studies have established that pro-inflammatory cytokines are powerful modulators of neuroendocrine function. TNF-α, in particular, can cross the blood-brain barrier or act on circumventricular organs to suppress the pulsatile release of Gonadotropin-Releasing Hormone (GnRH) from the hypothalamus.[9] Furthermore, cytokines can directly inhibit pituitary gonadotroph cells, blunting their response to GnRH and thereby suppressing the secretion of LH necessary to stimulate testicular testosterone production. This creates a compelling pathophysiological link between the immune dysregulation of HIV and the endocrine failure of the HPG axis.

However, the vast majority of contemporary research on HIV comorbidities is conducted in populations receiving ART. These invaluable studies are inherently confounded by the complex effects of antiretroviral drugs, which can have their own metabolic and immunomodulatory impacts. The ART-

naïve population, while representing a diminishing demographic globally, offers a unique and scientifically crucial human model. Studying these individuals provides an unfiltered view into the direct pathophysiological consequences of uncontrolled viremia and the host immune response, free from therapeutic intervention. Such investigations are essential for elucidating the foundational mechanisms of HIV-related organ damage.[10]

Therefore, this study was designed to perform a detailed immuno-endocrine investigation in a well-characterized cohort of ART-naïve men with HIV in Palembang, Indonesia. The primary novelty and aims of this study were threefold: (1) to determine the prevalence and type (primary vs. secondary) of hypogonadism in this unique population; (2) to quantify the relationship between serum TNF-α, as a cardinal marker of HIV-associated inflammation, and the functional status of the HPG axis, specifically testosterone and LH levels; and (3) to utilize multivariate analysis to explore the independent associations of inflammation (via TNF-α) and nutritional status (via body mass index) with testosterone deficiency, thereby dissecting the interplay of these two critical factors. We hypothesized that secondary (hypogonadotropic) hypogonadism would be the predominant form of testosterone deficiency and that its severity would be independently associated with both higher levels of systemic inflammation and poorer nutritional status.

2. Methods

This investigation was conducted as an analytical, observational study with a cross-sectional design at the HIV outpatient clinic of Dr. Mohammad Hoesin General Hospital, a tertiary referral center in Palembang, South Sumatra, Indonesia. Patient recruitment and data collection were performed between April 2024 and July 2024. The study population comprised adult male patients with a confirmed HIV diagnosis who had never received antiretroviral therapy (ART-naïve). A consecutive sampling method was employed to recruit all eligible and consenting patients during the study period. Inclusion criteria were: (1) confirmed HIV-1 infection via national standard serological testing protocols; (2) male sex, aged between 18 and 60 years; (3) documented ART-naïve status; and (4) provision of written informed consent. Exclusion criteria were rigorously established to minimize the influence of confounding factors known to independently affect the HPG axis or inflammatory status. These included: (1) known pre-existing endocrine disorders, including pituitary adenoma, Klinefelter syndrome, or prior orchiectomy; (2) uncontrolled diabetes mellitus, defined as HbA1c > 8.0%; (3) severe chronic kidney disease (estimated Glomerular Filtration Rate < 30 mL/min/1.73m²) or end-stage renal disease; (4) decompensated liver cirrhosis (Child-Pugh Class B or C); (5) active hematological or solid organ malignancy; (6) diagnosed systemic autoimmune diseases such as systemic lupus erythematosus or rheumatoid arthritis; (7) current or recent (within 3 months) use of medications known to affect testosterone levels or the HPG axis, such as glucocorticoids, opioids, anabolic steroids, GnRH analogs, or other immunosuppressants; and (8) an acute, severe non-HIV-related illness requiring hospitalization at the time of enrollment.

An a priori sample size calculation was performed based on detecting a significant correlation between TNF-α and total testosterone, as suggested by prior literature. Assuming a moderate effect size (Pearson's r = 0.40), a two-tailed alpha of 0.05, and a desired statistical power of 80%, the required sample size was calculated to be 47 participants. Acknowledging the challenge of recruiting this specific ART-naïve population, we aimed for a target of at least 40 participants. A post-hoc power analysis on our final sample of 40 subjects, given the observed Spearman's correlation coefficient of $\rho = -0.411$, confirmed that the study achieved a statistical power of approximately 85% to detect the primary association of interest.

The study protocol received full approval from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Sriwijaya, and Dr. Mohammad

Hoesin General Hospital. The research was conducted in strict accordance with the ethical principles outlined in the Declaration of Helsinki. All potential participants were engaged in a detailed informed consent process, during which the study's purpose, procedures, potential risks, and benefits were thoroughly explained in the local language. Written informed consent was obtained from every individual prior to enrollment and any study-related activities. To ensure confidentiality, all participant data and biological samples were de-identified and assigned a unique study code.

Upon enrollment, each participant underwent a comprehensive clinical evaluation by a study physician. Sociodemographic data (age, education, employment) and a detailed medical history, including HIV risk factors, were collected using a standardized case report form. The presence and severity of symptoms commonly associated with testosterone deficiency were systematically assessed using a structured questionnaire adapted from established clinical tools, querying for erectile dysfunction, depressive symptoms, and insomnia.

A physical examination was conducted, including measurement of height using a stadiometer (to the nearest 0.1 cm) and weight using a calibrated digital scale (to the nearest 0.1 kg). Body Mass Index (BMI) was calculated as weight (kg) / height (m)^2. BMI was categorized according to WHO criteria for Asian populations: underweight (<18.5 kg/m^2), normal weight (18.5–22.9 kg/m^2), overweight (23.0–24.9 kg/m^2), and obese (≥25.0 kg/m^2). The clinical stage of HIV infection was determined according to the 2007 World Health Organization (WHO) clinical staging system.

Fasting venous blood samples (10 mL) were collected from each participant between 07:00 and 09:00 AM to minimize variability from the diurnal rhythm of testosterone secretion. Blood was drawn into serum-separating tubes and EDTA tubes. Serum was separated by centrifugation at 3000 rpm for 15 minutes within one hour of collection. Aliquots of serum and plasma were immediately stored at -80°C until they were analyzed in batches to reduce inter-assay variability.

Hormonal and inflammatory marker analysis

Total Testosterone (TT): Serum TT levels were measured using a competitive enzyme-linked immunosorbent assay (ELISA) kit (Cat# DKO001, DiaMetra, Italy). The assay's detection range is 0.05-16 ng/mL. For consistency, original units of pg/mL are reported, with a normal reference range considered >3000 pg/mL (equivalent to 3.0 ng/mL or 300 ng/dL); Luteinizing Hormone (LH): Serum LH levels were measured using a sandwich ELISA kit (Cat# E-EL-H0077, Elabscience, USA) with a detection range of 0.78-50 mIU/mL. The laboratory's normal reference range is 1.7–8.6 mIU/Ml; Tumor Necrosis Factor-alpha (TNF-α): Serum TNF-α concentrations were quantified using a high-sensitivity sandwich ELISA kit (Cat# KHC3014, Invitrogen, USA) with a lower limit of detection of 0.17 pg/mL and a quantitative range up to 10.8 pg/mL. Samples with higher concentrations were diluted according to the manufacturer's protocol; Interleukin-6 (IL-6): Serum IL-6 was measured using a sandwich ELISA kit (Cat# ab46027, Abcam, USA) with a detection range of 1.56-100 pg/mL; High-Sensitivity C-Reactive Protein (hs-CRP): Serum hs-CRP was measured using a particle-enhanced immunoturbidimetric assay on an automated clinical chemistry analyzer.

Virological and hematological analysis

HIV-1 Viral Load (VL): Plasma HIV-1 RNA was quantified using a real-time polymerase chain reaction (RT-PCR) assay (Abbott RealTime HIV-1, Abbott Molecular, USA), with a lower limit of detection of 40 copies/mL; Routine Laboratory Tests: A complete blood count (CBC), renal function tests (urea, creatinine), and liver function tests (SGOT, SGPT) were performed using standard automated methods at the hospital's central laboratory.

Definition of hypogonadism

Hypogonadism was biochemically defined using fasting morning total testosterone levels according to the Endocrine Society clinical practice guidelines. A diagnosis was made if the total testosterone level was below 3000 pg/mL (3.0 ng/mL). The type of hypogonadism was then classified based on the corresponding LH level: Primary (Hypergonadotropic) Hypogonadism: Low total testosterone (<3000 pg/mL) with an elevated LH level (>9.4 mIU/mL, the upper limit of the normal reference range); Secondary (Hypogonadotropic) Hypogonadism: Low total testosterone (<3000 pg/mL) with a low or inappropriately normal LH level (≤9.4 mIU/mL).

Statistical Analysis

All statistical analyses were conducted using SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). The normality of distribution for continuous variables was assessed using the Shapiro-Wilk test. As key variables (testosterone, LH, TNF-α, viral load, BMI) were not normally distributed ($p < 0.05$), non-parametric methods were used for inferential statistics, and data were presented as median and interquartile range (IQR). Descriptive statistics were used to summarize cohort characteristics, with categorical variables presented as frequencies and percentages (%). Bivariate relationships were assessed using Spearman's rank correlation coefficient (ρ). The strength of correlation was interpreted as: $\rho < 0.3$ (weak), $\rho = 0.3–0.5$ (moderate), and $\rho > 0.5$ (strong).

To investigate the independent predictors of testosterone deficiency, a multiple linear regression analysis was performed. Due to its skewed distribution, the total testosterone level was natural log-transformed (Ln-Testosterone) to better approximate a normal distribution for the model's residuals. Ln-Testosterone was set as the dependent variable. Serum TNF-α and BMI were entered as independent variables. Standard checks for the assumptions of linear regression were performed, including linearity, independence of residuals (Durbin-Watson test), homoscedasticity, and multicollinearity (Variance Inflation Factor, VIF). A two-tailed p-value of < 0.05 was considered statistically significant for all analyses.

3. Results

A total of 40 ART-naïve men with HIV were enrolled. The baseline demographic and clinical characteristics are detailed in Table 1. The cohort had a mean age of 35.6 ± 10.2 years. The predominant risk factor for HIV transmission was homosexual contact (67.5%, n=27). The clinical profile was indicative of advanced immunosuppression. The vast majority of subjects were classified as WHO Clinical Stage III (77.5%, n=31), with the remainder in Stage IV (22.5%, n=9). A profound nutritional deficit was evident, with half of the cohort (50.0%, n=20) classified as underweight (BMI < 18.5 kg/m²). Clinical symptoms of testosterone deficiency were nearly universal. Ninety percent of participants (n=36) reported experiencing both erectile dysfunction and depression, and 75.0% (n=30) reported insomnia. Every participant reported at least one major symptom of hypogonadism.

The cohort's laboratory profile, summarized in Table 2, confirmed high-level viremia, profound hypogonadism, and severe systemic inflammation. The median HIV-1 viral load was 244,000 copies/mL (IQR: 85,500–765,000), indicating uncontrolled viral replication. A striking finding was the universal prevalence of hypogonadism; 100% of participants (40/40) met the biochemical criteria. The median total testosterone level was exceptionally low at 6.48 pg/mL (IQR: 3.15–15.80). Despite this profound gonadal failure, the pituitary response was blunted, with a median serum LH of 3.86 mIU/mL (IQR: 1.98–6.55), which is within the normal range. Consequently, all 40 cases were classified as secondary (hypogonadotropic) hypogonadism, with no instances of primary hypogonadism observed. Consistent with uncontrolled HIV infection, inflammatory markers were markedly elevated. The median serum TNF-α level was 10.32 pg/mL (IQR: 4.55–22.15), well above the healthy reference range. Similarly, median IL-6

and hs-CRP levels were high at 8.9 pg/mL and 12.5 mg/L, respectively.

Routine hematological and biochemical parameters are presented in Table 3. The data reflect the systemic impact of advanced HIV disease, with a high prevalence of anemia (mean hemoglobin: 9.73 ± 1.82 g/dL) and immune dysregulation.

Table 1. Demographic and Clinical Characteristics

A detailed breakdown of the study population (N=40).

VARIABLE	CATEGORY / VALUE	COUNT (N)	PERCENTAGE (%)
Age (years)	**Mean ± SD**	35.6 ± 10.2	-
	18–30	14	35.0%
	31–40	13	32.5%
	41–50	5	12.5%
	51–60	8	20.0%
Body Mass Index (kg/m²)	Underweight (<18.5)	20	50.0%
	Normal (18.5–22.9)	13	32.5%
	Overweight (23.0–24.9)	4	10.0%
	Obese (≥25.0)	3	7.5%
Clinical Profile Symptoms of Testosterone Deficiency & WHO Stage	Erectile Dysfunction	36	90.0%
	Depression	36	90.0%
	Insomnia	30	75.0%
	WHO Stage III	31	77.5%
	WHO Stage IV	9	22.5%

All data based on the total study population of N=40 ART-naïve men with HIV.

Table 2. Hormonal, Virological, and Inflammatory Characteristics

A summary of key biomarkers for the study population (N=40).

Universal Secondary Hypogonadism

A striking **100%** of participants (40 out of 40) were diagnosed with secondary (hypogonadotropic) hypogonadism, indicating a central defect in the HPG axis rather than primary testicular failure.

Virological Profile

HIV-1 Viral Load (copies/mL)

Median (IQR):	244,000 (85k – 765k)
Mean ± SD:	788,697 ± 1.96M
Range:	40 – 12,000,000

Hormonal Profile

Total Testosterone (pg/mL)

Median (IQR):	6.48
Range:	1.25 – 50.20

Luteinizing Hormone (LH) (mIU/mL)

Median (IQR):	3.86 (Normal)
Range:	0.18 – 16.54

Inflammatory Markers

TNF-α (pg/mL)
10.32 Median

IL-6 (pg/mL)
8.9 Median

hs-CRP (mg/L)
12.5 Median

*BDL: Below Detection Limit. Three samples had TNF-α levels below the assay's lower limit of detection (0.17 pg/mL).

Table 3. General Hematological & Biochemical Parameters

An overview of routine laboratory results for the study population (N=40).

Hematology

Blood count and cell parameters

	Mean ± SD:	Median (Range):
Hemoglobin (g/dL)	9.73 ± 1.82	9.15 (6.6–14.9)
Leukocyte (x10³/mm³)	5.85 ± 2.15	5.71 (1.12–26.39)
Platelet (x10³/mm³)	221.50 ± 145.80	193.50 (111–670)
Neutrophil (%)	66.5 ± 23.07	73.0 (7–97)
Lymphocyte (%)	22.05 ± 19.47	16.0 (2–88)

Biochemistry

Renal and liver function

	Mean ± SD:	Median (Range):
Urea (mg/dL)	36.9 ± 21.32	34.0 (12–116)
Creatinine (mg/dL)	0.85 ± 0.26	0.80 (0.52–2.05)
SGOT (U/L)	48.85 ± 36.82	38.5 (11–203)
SGPT (U/L)	40.98 ± 36.14	33.0 (7–224)

Spearman's rank correlation analysis was performed to assess the relationships between immuno-endocrine variables (Table 4 and Figure 1). A statistically significant, moderate negative correlation was found between serum TNF-α and total testosterone levels (ρ = -0.411, p = 0.008) (Figure 1). This indicates that higher levels of TNF-α were associated with lower testosterone. In contrast, there was no significant correlation between TNF-α and LH (ρ = 0.152, p = 0.351), highlighting the inadequate pituitary response. Confirming the link between viral activity and inflammation, HIV-1 viral load demonstrated a significant positive correlation with TNF-α (ρ = 0.485, p = 0.001), IL-6 (ρ = 0.512, p < 0.001), and hs-CRP (ρ = 0.450, p = 0.003).

To disentangle the effects of inflammation and malnutrition on testosterone levels, a multiple linear regression analysis was conducted. The model, with log-transformed total testosterone as the dependent variable, was statistically significant ($F(2, 37) = 11.25$, $p < 0.001$) and explained 34.6% of the variance in testosterone levels (Adjusted $R^2 = 0.346$). As shown in Table 5, both serum TNF-α and BMI were significant, independent predictors of testosterone. After controlling for BMI, higher TNF-α levels remained significantly associated with lower testosterone (β = -0.38, p = 0.011). Similarly, after controlling for TNF-α, a lower BMI (indicating worse nutritional status) was independently associated with lower testosterone (β = 0.45, p = 0.003). The standardized coefficients (β) suggest that BMI had a slightly stronger independent association with testosterone levels in this cohort compared to TNF-α.

Table 4. Spearman's Rank Correlation Matrix

Heatmap of key immuno-endocrine variable relationships.

	HIV-1 VL	Testosterone	LH	TNF-α	IL-6	hs-CRP
HIV-1 VL	1.000	-0.345	0.098	0.485	0.512	0.450
Testosterone	-0.345	1.000	0.055	-0.411	-0.389	-0.315
LH	0.098	0.055	1.000	0.152	0.180	0.112
TNF-α	0.485	-0.411	0.152	1.000	0.645	0.589
IL-6	0.512	-0.389	0.180	0.645	1.000	0.510
hs-CRP	0.450	-0.315	0.112	0.589	0.510	1.000

Correlation (ρ): -1.0 +1.0 **Bold**: p < 0.01 *Italic*: p < 0.05

How to Read This Heatmap

This matrix visualizes the strength and direction of relationships between variables. Each cell shows the Spearman's correlation coefficient (ρ). **Stronger red colors** indicate stronger positive correlations (as one variable increases, the other tends to increase). **Stronger blue colors** indicate stronger negative correlations (as one variable increases, the other tends to decrease). **White or neutral colors** indicate a weak or no correlation. Hover over a cell to see the corresponding p-value.

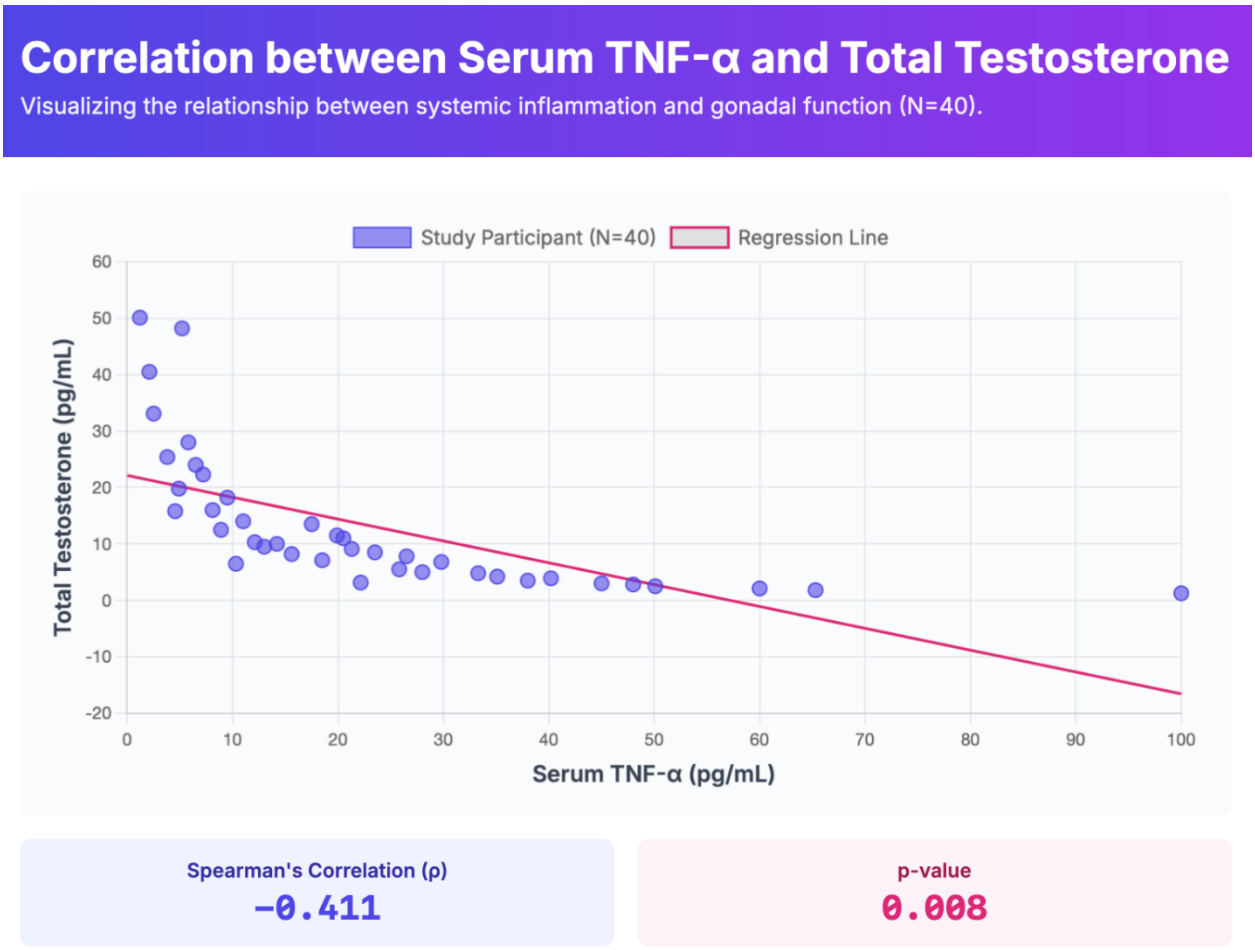

Figure 1. Correlation between serum TNF-α and total testosterone.

4. Discussion

This study provides a detailed immuno-endocrine characterization of ART-naïve men with advanced HIV, yielding several critical insights into the pathophysiology of HIV-associated hypogonadism. The primary findings are: (1) a universal (100%) prevalence of hypogonadism, which was exclusively secondary (hypogonadotropic) in nature; (2) a significant inverse correlation between the pro-inflammatory cytokine TNF-α and total testosterone levels; and (3) a novel demonstration through multivariate analysis that both systemic inflammation (TNF-α) and malnutrition (BMI) are strong, independent statistical predictors of testosterone deficiency in this population.[11]

The most unequivocal finding of our study is the uniform presence of secondary hypogonadism in all 40 participants.[12] In a healthy state, the profound testosterone deficiency observed (median 6.48 pg/mL) should elicit a robust compensatory feedback signal to the pituitary, resulting in a surge of LH secretion to stimulate the testes.

Table 5. Multiple Linear Regression Analysis

Predicting Log-Transformed Total Testosterone (N=40)

Model Summary

Adjusted R^2	F-statistic	Degrees of Freedom	Model p-value
0.346	11.25	2, 37	<0.001
34.6% of variance explained			Highly Significant

Independent Predictors

VARIABLE	COEFFICIENT (B)	95% CI FOR B	BETA (B)	T-VALUE	P-VALUE
(Constant)	1.15	(0.28, 2.02)	–	2.68	0.011
Serum TNF-α (pg/mL)	−0.018	(−0.031, −0.004)	**−0.380**	−2.67	0.011
Body Mass Index (kg/m²)	+0.065	(0.024, 0.106)	**0.451**	3.16	0.003

Interpretation of the Model

The multiple regression model was statistically significant ($p < 0.001$) and explains **34.6%** of the variability in log-transformed total testosterone levels (Adjusted $R^2 = 0.346$). Both inflammation and nutritional status were found to be independent predictors:

- **Serum TNF-α:** For every 1 pg/mL increase in TNF-α, the log-transformed testosterone level is predicted to decrease by 0.018, holding BMI constant. This effect is statistically significant ($p = 0.011$).
- **Body Mass Index:** For every 1 unit increase in BMI, the log-transformed testosterone level is predicted to increase by 0.065, holding TNF-α constant. This effect is also statistically significant ($p = 0.003$).

The Standardized Coefficient (Beta) suggests that BMI ($\beta = 0.451$) had a slightly stronger relative influence on testosterone levels in this model compared to TNF-α ($\beta = -0.380$).

The consistent absence of this response—manifested as inappropriately normal LH levels—points decisively to a functional defect within the central components of the HPG axis (the hypothalamus and/or pituitary) rather than to primary testicular failure.[13] While previous studies have reported secondary hypogonadism as the predominant form, the 100% prevalence seen here is exceptional. This powerfully suggests that in the setting of advanced, untreated HIV, direct viral orchitis or damage from opportunistic pathogens are not the principal etiologies of testosterone loss.[14] Instead, our data provide compelling clinical evidence for a state of "functional acquired hypogonadotropic hypogonadism," a condition driven not by structural pathology but by the potent suppressive effects of a severe systemic illness (Figure 2).

Our findings strongly implicate chronic inflammation as a key physiological process associated with this central HPG axis suppression. The significant negative correlation between TNF-α and total testosterone provides a quantitative link between the inflammatory state and the endocrine outcome.[15] This statistical association is grounded in well-established biological mechanisms. Pro-inflammatory cytokines, including TNF-α and IL-1, are

known to be powerful disruptors of the delicate neuroendocrine regulation of reproduction.[16] These molecules can directly influence the central nervous system by crossing the blood-brain barrier or acting on circumventricular organs, which lack a complete barrier.

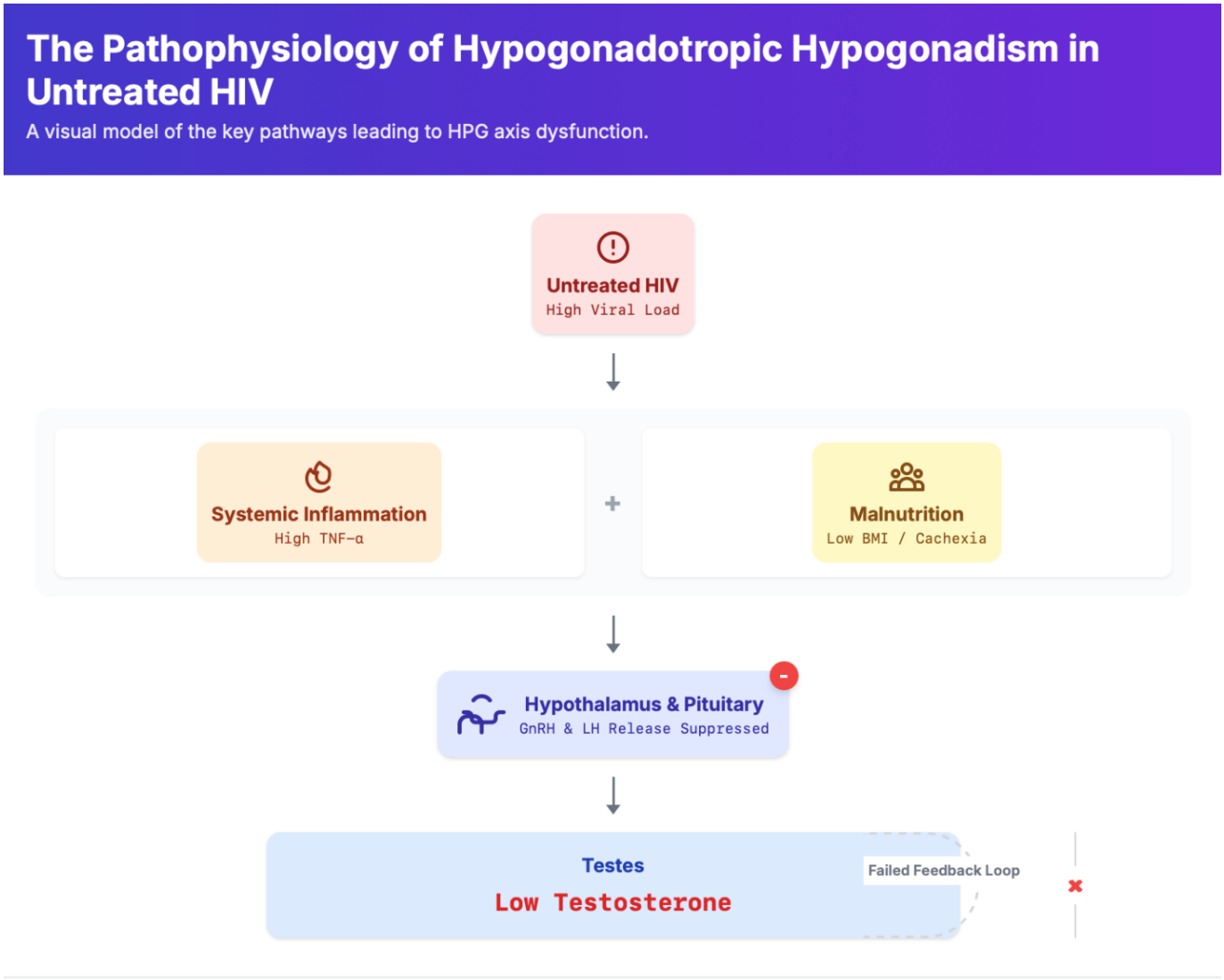

Figure 2. The centrality of hypogonadotropic hypogonadism in untreated HIV.

At the hypothalamic level, TNF-α has been shown to inhibit the intricate pulsatile secretion of GnRH, the master regulator of the HPG axis.[17] At the pituitary level, cytokines can exert direct inhibitory effects on gonadotroph cells, blunting their responsiveness to GnRH stimulation and thereby suppressing LH release. The biochemical signature of our cohort—low testosterone, inappropriately normal LH, and markedly elevated TNF-α—is the precise clinical manifestation of this cytokine-associated central suppression. The strong positive correlation between HIV-1 viral load and TNF-α ($\rho = 0.485$) completes the proposed pathophysiological pathway: uncontrolled viral replication is associated with systemic inflammation, which in turn is associated with central suppression of the HPG axis, culminating in hypogonadism.

A novel contribution of this study is the use of multivariate regression to statistically dissect the relative contributions of inflammation and malnutrition to testosterone deficiency. The bivariate analysis showed a moderate correlation between TNF-α and testosterone, explaining approximately 17% of the variance ($\rho 2 = 0.169$), which correctly implies that TNF-α is a significant, but not the sole, factor involved. Malnutrition and cachexia are also potent, independent causes of functional hypogonadotropic hypogonadism, representing an adaptive physiological response to conserve energy during periods of severe catabolism by down-regulating non-essential functions like reproduction.

Our multiple regression model provided crucial clarity on this interplay. The analysis demonstrated that even after statistically controlling for the potent effect of BMI, TNF-α remained a significant, independent predictor of testosterone levels. This finding strengthens the hypothesis that systemic inflammation has a direct, suppressive effect on the HPG axis, independent of its contribution to weight loss. Conversely, a lower BMI was also a powerful independent predictor of lower testosterone, even when the effect of TNF-α was held constant.[18] This confirms that malnutrition itself is a major contributor to hypogonadism in this setting. Together, these two factors explained over a third (34.6%) of the total variance in testosterone levels. This suggests a "two-hit" model, where the clinical picture of severe hypogonadism in our cohort is best understood as the result of two distinct but synergistic insults: a direct, cytokine-mediated suppression of the HPG axis and a parallel, malnutrition-induced adaptive shutdown of reproductive function.

This profound biochemical derangement is not a benign laboratory finding; it has severe clinical consequences that were overwhelmingly evident in our cohort.[19] The near-universal reporting of erectile dysfunction (90%), depression (90%), and insomnia (75%) aligns perfectly with the known clinical sequelae of severe testosterone deficiency. Hypogonadism is an independent contributor to fatigue, loss of libido, and mood disorders, and its presence likely exacerbates the already immense burden of disease in these individuals. Furthermore, testosterone is a potent anabolic hormone. Its deficiency contributes directly to the loss of muscle mass and lean body tissue, which are hallmarks of the AIDS wasting syndrome. This establishes a potential vicious cycle: HIV drives inflammation, which, along with malnutrition, causes hypogonadism. The resultant low testosterone then exacerbates muscle wasting and potentially worsens mood and overall health status, further compromising the host's ability to combat the underlying disease. These findings powerfully illustrate that the observed immuno-endocrine dysregulation is a central component of the clinical syndrome of advanced, untreated HIV.[20]

While this study provides a clear and compelling picture, its limitations must be acknowledged. The primary limitation is its cross-sectional design, which demonstrates strong associations but cannot establish causality or determine the temporal sequence of events. Longitudinal studies are required to confirm that inflammation and malnutrition directly cause a decline in HPG axis function over time. Secondly, the use of consecutive sampling, while pragmatic, is a non-probability method that may

introduce selection bias and limit the generalizability of our findings to all ART-naïve populations. Finally, our sample size, while adequately powered for our primary analysis, was modest and may have been insufficient to detect weaker, but still potentially relevant, associations or to build more complex predictive models.

5. Conclusion

In conclusion, this study reveals a universal prevalence of secondary (hypogonadotropic) hypogonadism among ART-naïve men with advanced HIV, indicating a profound and consistent failure of the central hypothalamic-pituitary-gonadal axis. Our findings provide strong evidence that this endocrine dysfunction is independently associated with two powerful biological insults: the severe systemic inflammation intrinsically linked to uncontrolled viral replication, with TNF-α as a key correlate, and the concurrent state of severe malnutrition. This work elucidates a critical pathophysiological model where inflammation and cachexia act as distinct, synergistic partners in the suppression of the male reproductive axis. These results underscore the importance of addressing both the inflammatory state and nutritional status in the comprehensive management of HIV, and they provide a compelling rationale for the routine screening of hypogonadism as an integral part of the initial clinical assessment of all men newly diagnosed with HIV.

6. References

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