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Vitamin A deficiency in human immunodeficiency virus Type-1 infected and un-infected pregnant women in KPK, Pakistan

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Abstract

Background: Vitamin A deficiency (VAD) is a major public health problem in developing countries and a large number of pregnant women, especially in low-income communities, are more susceptible to VAD. This study aimed to report the baseline levels of Vitamin A in pregnant women with and without HIV-1 infection and to ascertain the association of CD4 + count and viral load with vitamin A deficiency. Methodology: A cross-sectional study was conducted in which HIV-1 infected and uninfected pregnant women were recruited. 24hr Dietary recall data were obtained using Windiest software. CD4 T cell counts in HIV-1+ patients were measured using a Pima Analyzer. Serum Vitamin A levels were performed using Enzyme Linked immune-sorbent Assay (ELISA). Results: The mean age of the participants was 27.05± 5.98. The mean Vitamin A levels were 92.41±57.32, and the mean CD4+ count was 631.78±271.29. In the case and control groups, vitamin A levels showed a statistically significant difference (p =0.003). No significant correlation was observed among the VAD, viral load (P=0.79), and CD4+ count (P=0.84). Conclusion: This study concluded that HIV-1-positive pregnant women often suffer from vitamin A insufficiency, and supplementation with vitamin A and other micronutrients is crucial for improving their health outcomes.

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Introduction

Vitamin A deficiency (VAD) is a serious nutrient shortage in impoverished communities, particularly in low-income countries, and the majority of affected women of reproductive age are from Africa and Southeast Asia (Imdad A, *et al.,* 2017, Organization WH, 2009). The VAD has been linked to pregnancy and HIV infection. Owing to the increased demand for vitamin A by the developing fetus, pregnant women are at a major risk of VAD. Vitamin A is transferred from mother to infant through the placenta during pregnancy, birth, and lactation (Azaïs-Braesco V, *et al.,* 2000). When there is an acutely low vitamin A dietary intake, a sustained period of dietary scarcity, or a concurrent blend of two conditions, with the probable arbitration of an underlying illness, a typical pattern involving VAD occurs (Organization WH, 2009). VAD may worsen infection-related birth canal abrasion, which can lead to high exposure to HIV-infected inflammatory cells. VAD may enhance the likelihood of intrapartum transmission by increasing titers of the virus in the blood, thus increasing vulnerability during labor and delivery. VAD might increase the number of cells infected by HIV-disrupting immunity of mothers in breast milk and might lower HIV-specific IgA-antibody titers, leading to infant HIV exposure (Stephensen CB, *et al.,* 2003).

In some cultures, night blindness, the main diagnostic demonstration of VAD, is widespread among pregnant women and is regarded as a symptom of pregnancy along with morning sickness (Raman L, *et al.,* 1991). HIV-associated opportunistic infections may result in generalized inflammation, which can lead to impaired mobilization of Vitamin A liver despite sufficient reserves of vitamin A, along with abnormal urinary loss of vitamin A (Stephensen CB, *et al.,* 1994). Vitamin deficiency in pregnant women can weaken immunity and promote multidrug resistance, underscoring the critical role of proper nutrition in mitigating these risks during pregnancy. HIV-positive pregnant women show a decline in CD4+ count due to hemodilution during gestation, which becomes normal after delivery (Burns DN, *et al.,* 1996).

This is the first study of its kind to be carried out in Pakistan, and it measures the levels of vitamin A in pregnant women who are HIV-positive and HIV-negative. Both HIV infection and vitamin A deficiency are highly prevalent in many resource-limited settings. The identification of micronutrient levels is important to reduce mortality and morbidity in the search for cost-effective interventions, such as vitamin A supplements. Studies related to the effects of vitamin A status on functional immunity have not been conducted in participants with HIV infection in this region. This study aimed to assess the baseline levels of vitamin A in pregnant women, CD4+ count, and viral load associated with vitamin A deficiency.

Materials and methods

Participants and study design

The purpose of this cross-sectional study was to evaluate vitamin A levels in pregnant women. All potential participants were divided into two groups: case and control. This study was conducted over nine months, from October 2020 to June 2021. Data collection and sample analyses were performed during this period. Pregnant women who visited the Hayatabad Medical Complex (HMC) for routine antenatal care follow-up were recruited. Only (n=18) HIV-infected pregnant women with HIV infection attending the family care center were eligible for the study. For each case, four healthy HIV-negative controls living in the same geographic location were recruited in the study. The control group (n= 69) was comprised of healthy pregnant women with HIV infection. Ethical approval was obtained from the institutional ethics committee. None of the HIV-1+ patients had a co-infection or any other comorbidity and were not taking Vitamin A supplements.

After obtaining written informed consent, interviews and 24-hour dietary recall were conducted. The intake of food and beverages was recalled, described, and quantified precisely in 24-hour time during the day and prior to the interview. Twenty-four-hour data were recorded from the first intake of food in the morning to the last intake at night. Windiet software version 2005 is computer software that was used to convert the collected information into data.

Body height and weight were measured by using a height stadiometer (ZT-120). Anthropometric data were measured according to the standard method, and the body mass index (BMI) was calculated.

Biochemical Analysis

Following the Chemiflex protocols, HIV-1 serology was carried out using the Architect i2000 SR and the Architect HIV Ag/Ab Combo assays, a two-step CMIA for identifying antibodies to HIV-1, HIV-2, and p24 antigen. HIV-1 DNA results were obtained from the patient's medical records, with RNA amplification achieved through Polymerase Chain Reaction (PCR) within a quantifiable range of 20 copies/mL.

CD4 cell count was measured using a Pima Analyser (Alere- 03858). The Pima Analyzer is a portable bench-top fixed volume cytometer used for the processing and analysis of a Pima test cartridge and uses LED illumination and a CCD-based detection system. Serum Vitamin A levels were performed using Enzyme Linked immune-sorbent Assay (ELISA).

Statistical Analysis

During the study duration, all important data were consistently recorded in Microsoft Excel® 2013. After collecting, organizing, and coding all the results in Excel, they were imported into IBM SPSS® (Version 22) for statistical analysis. An independent sample T test was performed to compare the differences. Mann Whitney U test was applied to compare means between case and controls. Results with a p-value less than 0.05 were considered statistically significant. For the correlation among variables, bivariate Pearson correlation was used.

Results

The mean age of the participants was 27.05± 5.98. All participants (n=87) were married, and their gestational age was ranging from to 24-39 weeks. The major proportion was non-anemic with no drug addiction. Among all subjects, 40.2% had a normal BMI, 25.3% were overweight, and 34.5% were obese. Among these, 38.8% had a normal BMI compared to controls (40.5%). of eighty-seven participants, 81.6% had multigravida pregnancies and 18.4% had primigravida pregnancies. In our study, 48.3% of the participants had two or more children, whereas 26.4% had one child. Only 2.3% had used needles, and 3.4% reported a hospitalization history. All HIV-positive patients received combination antiretroviral therapy containing 3TC + TDF + EFV. The general characteristics of the participants are presented in Table 1.

Twenty hours of dietary recall data of the participants are presented in Table 2. Mean and standard deviation was calculated for all dietary assessment variables. Overall, energy intake was slightly higher than the energy intake required for the third trimester.

Significant differences were observed between the case and control for the intake of sugars (g), water, copper (mg), and retinol (µg). CD4+ T cell counts were measured in all HIV-positive patients who visited the FCC during the study period.

The mean serum vitamin A was found to be 92.41 (57.32) in all participants (n=87). A maximum CD4 + count of 1084 was also reported in this study. All patients adhered to antiretroviral therapy; therefore, their CD4+ count was above 500 cells/mm^3 in 66.6% of the patients. Viral Load data were obtained from the patient's medical history with PCR performed within the last 6 months of the study. The mean CD4+ count was 631.78± 271.29 and the average viral load was 12.23± 22.53.

A correlation test was run to determine the association between Serum levels of Vitamin A and dietary intake of Vitamin A. There was no statistically significant association between these variables (r= 0.09, p = 0.37). To determine the correlation between Vitamin A with CD4 T-cell counts, Bivariate Pearson correlation analysis was performed using SPSS.

No statistically significant correlation was observed between the CD4 T-cell count and vitamin A levels (r=0.04, p=0.84) (Fig 3.2). The correlation coefficient between viral load and vitamin A levels was -0.67 (p-value= 0.79) (Fig 3.3).

Table 1. General characteristics of all the participants (n=87).

Characteristics	Frequency (n)	Percent (%)
Age		
<20	18	20.7
21-30	51	58.6
31-40	18	20.7
Drug Addict		
Yes	1	1.1
No	86	98.9
Nutritional status		
Normal Weight	35	40.2
Overweight	22	25.3
Obesity	30	34.5
Number of Children		
None	22	25.3
1	23	26.4
≥2	42	48.3

Table 2. Detailed Dietary Recall analysis.

Factors	Case (n=18) Mean± SD	Control (n=69) Mean± SD	p-value
Energy KJ	4824.8 ± 2140	5663.47± 2097.9	0.160
Energy Kcal	1149.25 ± 509.09	1353.05 ± 509.09	0.153
CHO (g)	134.96 ± 66.44	157.74 ± 57.58	0.176
Protein (g)	40.23 ± 26.45	37.93± 19.09	0.946
Fat (g)	53.36 ± 25.11	66.67 ± 30.98	0.177
Sugars (g)	17.56 ± 8.68	28.49 ± 14.15	0.004*
Starch (g)	112.69 ± 57.2	123.95 ± 48.72	0.429
Water	489.31± 150.48	1141.5± 769.96	0.000*
Vitamins			
Vitamin A (µg)	386 ± 401.71	325.1± 279.61	0.743
Thiamine (mg)	0.83 ± 0.44	0.92 ± 0.40	0.447
Riboflavin (mg)	0.49 ± 0.26	0.60 ± 0.30	0.198
Niacin (mg)	22.15 ± 18	16.66± 8.	0.447
Vitamin B6 (mg)	0.92 ± 0.49	0.89 ± 0.38	0.826
Vitamin B12 (µg)	0.69 ± 1.25	0.68± 1.13	0.755
Folate (µg)	97.65 ± 71.47	125.69 ± 54.76	0.103
Pantothenic acid (mg)	1.96 ± 1.14	2.12± 1.20	0.766
Biotin (µg)	9.92 ± 4.86	14.94± 10.48	0.100
Vitamin C (mg)	17.02 ± 16.39	22.0± 23.66	0.838
Vitamin D (µg)	0.68 ± 1.15	0.56± 1.75	0.244
Vitamin E (mg)	9.1 ± 6.74	10.38 ± 5.09	0.405
Micronutrients			
Calcium (mg)	457.69 ± 287.07	542.13 ± 251.70	0.249
Iron (mg)	7.19 ± 4.19	7.71± 4.17	0.829
Zinc (mg)	4.91± 3.32	5.75± 3.61	0.383
Copper (mg)	0.64 ± 0.31	0.86 ± 0.36	0.02*
Manganese (mg)	2.83 ± 1.34	3.58 ± 1.47	0.06
Iodine (µg)	34.25 ± 31.54	34.597 ± 28.69	0.715
Retinol (µg)	39.44 ± 46.71	93.47± 96.94	0.005*
Carotene (µg)	1369.78 ± 2288.98	1014.2± 1413.13	0.470

Significant difference: *P < 0.05

Table 3 shows viral load analysis of HIV-infected patients. Viral load results revealed that 61.11% had not detected VL, 11.11% had VL copies below 20 and 22.22% had 20 or greater than 20 copies.

Table 3. Biochemical analysis of CD4 T-cell count and Viral load (for cases n=18 only).

Parameters	N	Mean	Standard Deviation
CD4+ Count	18	631.78	271.297
Viral Load	18	12.23	22.53

Table 4. Vitamin A deficiency in pregnant women by HIV serostatus.

Vitamin a status	With hiv	Without hiv
NON-VAD	1(5.55) *	3(4.34)
	273.10 ± 0**	233.73± 36.42
VAD	17(94.44)	65(94.20)
	57.44± 59.40	91.57± 41.08

*: N (%), **: Mean ± Standard deviation

Table 5. Comparison of Serum Vitamin A levels between Case and Control.

Serum	Sample Type	N	Mean	Std. Deviation	p-value
Vitamin A level	Control	69	98.41275	0.02674	0.003
	Cases	18	69.4310	76.84841	
	Difference		28.98		

Discussion

There is a major risk that the HIV/AIDS epidemic will continue to expand across the nation since it has firmly taken root. The 2011 National Nutrition Survey (NNS)(Ministry of Health Pakistan, 2011). concluded that deficiency of different micronutrients is an important public health issue, especially in pregnant ladies and the situation has worsened in 2018-2019 (Unicef. National Nutrition Survey 2018). In this study group receiving essential antenatal care, the observed prevalence of severe Vitamin A Deficiency (VAD) stands at 59.8%, which is considered a public health concern based on the WHO criterion of 15% VAD (Papathakis PC, *et al.,* 2007).

Another study conducted in KP reported the highest VAD prevalence of 76.2% (NACP. Ministry of National Health Services); however, the 2018 NNS report indicated a decline in VAD (Unicef. National Nutrition Survey 2018). Regardless of HIV status, biochemical evidence of VAD is common among antenatal women, which is in accordance with Andargachew *et al.,* (Mulu A, *et al.,* 2011). Overall, our findings revealed low concentrations of vitamin A (VA), regardless of HIV infection status.

Serum retinol levels below the cutoff of 200ng/mL are used to reflect insufficient VA levels as per NNS guidelines; however, biochemical low retinol concentration is still not believed to be a VAD, which is a limitation to compare findings. The VAD prevalence rates of 40% and 69% (Ahmed F, *et al.,* 2003) found in previous reports are somewhat closer to our results. Similar findings were reported by (Winit Phuapradit *et al.,* 2000) who endorsed the findings of our study. Reduced blood levels of carotene and retinol have also been observed in HIV-positive pregnant women, suggesting a link between these two factors. This relationship was explored in a study conducted by (H. Friis, *et al.,* 2001).

During the course of HIV infection, a decline in the release of Vitamin A liver, regardless of sufficient reserves of vitamin A in the liver, results in low serum retinol concentrations. The significantly higher VAD among HIV-infected females may be due to lower production of retinol-binding protein (RBP), which occurs because of the acute phase response to HIV infection (Suri DJ, *et al.,* 2021). No subclinical infection was confirmed in our study, as this study did not assess acute inflammatory responses; however, it should be noted that all participants showed up for routine antenatal follow-up at the time of blood collection. This deficiency can also be explained by liver homeostatic mechanisms, which act when a Vitamin A deficit results in the accumulation of hepatic RBP in hepatocytes. On the contrary, when the status of VA is sufficient, RBP is accumulated and synthesized at modest rates that lead to the release of very little or no freshly absorbed VA from the liver (Zanotti G *et al.,*, 2004). It has also been proposed that during pregnancy and postpartum, there are individual variations in hemodilution, and due to the elevated circulating plasma volume, it could lead to a decline in serum levels of retinol and its binding proteins (Kæstel P, *et al.,* 2012). WHO does not recommend supplementation of VA during pregnancy to reduce infant and maternal mortality and morbidity (McGuire S, *et al.,* 2011).

Association between the development of VAD and persistent decrease in $CD4^+$ cell levels has been reported by many studies (Semba RD, *et al.,* 1995) which contrasts with our study. This might be explained by a steady decline in $CD4^+$ count during pregnancy which returns to normal postpartum (Burns DN, *et al.,* 1996). The persistent drop in $CD4^+$ cell levels of the HIV-infected women suggests that HIV infection progresses during pregnancy and after delivery (Burns DN, *et al.,* 1996, Johnstone FD *et al.,* 1994) such decline is observed in 33% of cases in our study. An insignificant association was found between viral load and vitamin A levels, which is supported by a study carried out by (Winit Phuapradit*et al.,* 2000) who observed no significant correlation between Vitamin A levels and HIV-1 viral load.

One of the greatest breakthroughs in lowering MTCT rates in Pakistan has been developments in the Prevention of Parent to Child Transmission (PPTCT) program. Similar ART coverage was given to pregnant HIV females in our study, regardless of their CD4+ count Alvarez-Uria G *et al.,* 2012). The majority of HIV-positive patients are residing in the countryside. The observed difference may be contributed to a lack of knowledge of disease severity as well as improvidence. This finding contrasts with the work of (Mohammad *et al.,* 2014) who confirmed HIV acquisition to be associated with the urban population. Illiteracy was a common characteristic of HIV-positive patients. Out of all the study participants, 30.5% were anemic, out of which only 6 HIV- Positive cases were anemic. Anemia was also found in HIV patients in a study published in Colombia (Álvarez Barreneche MF, *et al.,* 2014) and in pregnant women in China (Hu YC, *et al.,* 2010). An increased risk of developing multi-drug resistance bacterial infections has been linked to lower vitamin A levels in HIV patients (Niaz F, *et al.,* 2022).

Limitations

The cross-sectional design, limited sample size, potential selection and recollection bias, lack of controls for confounding variables, and nutritional interactions are some of the study's drawbacks. Additionally, the study did not take into account additional medical issues, environmental and socioeconomic variables, or variations in laboratory

procedures, all of which may have impacted the generalizability and accuracy of the results.

Conclusion

Our research revealed a significant prevalence of vitamin A deficiency in pregnant HIV-1+ women, highlighting the need to supplement them with extra vitamin A and other essential micronutrients. We also stress the fact that couples who are in serious disorders have unique challenges and that the finest pregnancy management and good care are crucial to attaining the best results.

Potential conflicts of interest

All the authors declare no conflict of interest related to the manuscript content.

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