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SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE) PATTERN OF PRE-ANTI RETROVIRAL THERAPY(PRE ART) AND ANTI RETROVIRAL THERAPY(ART) TREATED MALE AND FEMALE HIV/AIDS PATIENTS

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ABSTRACT

Human immunodeficiency virus (HIV) causes **Acquired immune deficiency syndrome.** Human immune system is mainly affected by HIV. HIV transmitted through anal, vaginal or oral sex, blood transfusion, contaminated needles, exchange between mother and baby during pregnancy, breastfeeding, or other exposure to one of the above bodily fluids. Currently no vaccine is available for AIDS and HIV. Antiretroviral treatment reduces the mortality and the morbidity of HIV infection. HIV positive serum was collected from 5 group of patients. CD4 count was measured using flowcytometry. Serum proteins were separated by SDS – PAGE. In the SDS – PAGE, Alpha1 antitrypsin, Pre albumin, Albumin, Transferrin, Beta zone and Gamma zone was observed.3 year after the treatment of ART protein levels are increased in the male samples.

KEYWORDS: HIV, serum protein, ART, CD4 count.

INTRODUCTION

Human immunodeficiency virus (HIV) cause **Acquired immuno deficiency syndrome**. AIDS was first recognized by the U.S. (Centers for Disease Control and Prevention in 1981) causative organism of AIDS was identified in the early 1980. (Gallo RC (2006)). Young

people (under 25 years old) account for half of all new HIV infections worldwide. In developing and transitional countries, 9.7 million people were in immediate need of life-saving AIDS drugs; of these, only 2.99million (31%) are receiving the drugs.

DEFINITION

In 1993, the CDC expanded their definition of AIDS to include all HIV positive people with a $CD4^+$ T cell count below 200 per µL of blood or 14% of all lymphocytes. (The CDC, (1993), Retrieved on 2006-02-09).

Antiretroviral drugs

Antiretroviral drugs are medications for the treatment of infection by retroviruses, primarily HIV. They are not a cure for HIV. They lower the level of the virus in the blood. This allows the immune system to recover (the CD4 count may increase).People who are infected with HIV do not need antiviral treatment while their immune system is still strong (stages 1, 2 and 3).Antiretroviral treatment is only needed once the immune system has become weak. These medicines will not work effectively if they are not taken correctly.

Antiretroviral drugs

Zidovudin , Lamivudin, Zidovudin+ Lamivudin, Nevirapine.

MATERIALS AND METHODS

The present study entitled "**Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) pattern of Pre-Anti Retroviral Therapy(Pre ART) and Anti Retroviral Therapy(ART) treated male and female HIV/AIDS patients"** was carried out in the Department of Biochemistry and Microbiology, KAPV Government medical college, Trichy during March 2009 to August 2009.

Sample collection

Data on HIV infected patients were collected from ART centre of AGM Government hospital, Trichy. Before collecting the blood samples, explain the details about the project to the patients. Blood sample was collected from the interested candidates only. HIV-positive samples were collected with **universal precaution measures.**

Patients are categorized into 5 groups

1. Pre-ART-HIV-Positive patient not yet started with ART (55).
2. ART group 1- on treatment for 6months (6)

3. ART group 2- on treatment for 1year (11)
4. ART group 3- on treatment for 2 year (13)
5. ART group 4- on treatment for 3 year (10)

Two sets of samples will be collected

a. EDTA sample-CD4 count.

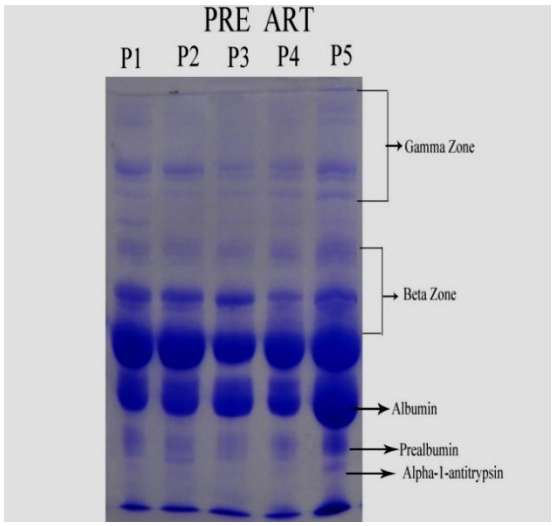
b. Clolted sample-SDS-PAGE

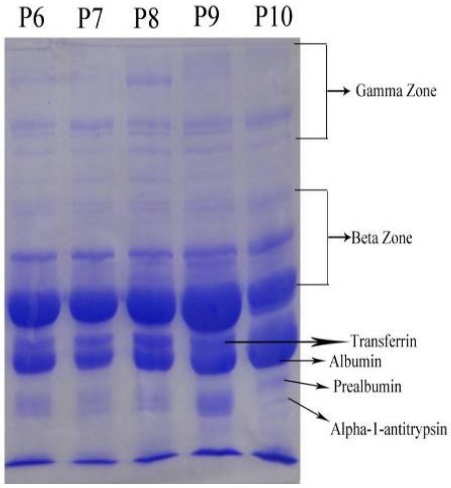
Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis analysis of Proteins

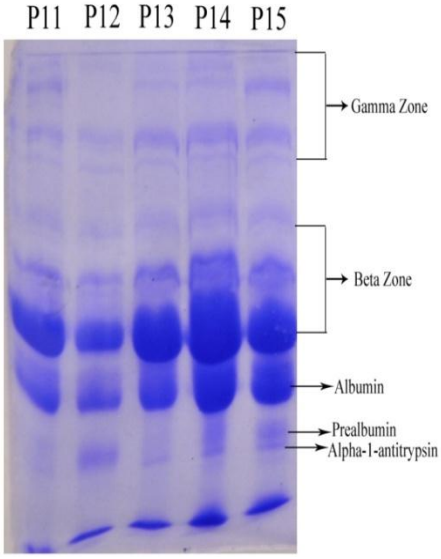
Solutions	Separating Gel Mixture	Stacking Gel Gel Mixture
Acrylamide monomer stock solution	10ml	1.33ml
4X Separating gel buffer,pH-8.8/4X Stacking gel buffer, pH-6.8	7.5ml	2.5ml
10% SDS solution	0.3ml	0.1ml
Distilled water	12.1ml	50µl
10% APS	150µl	5 µl
TEMED	10 µl	6.0ml

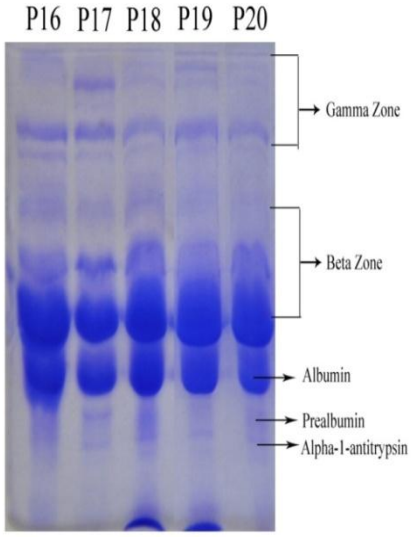
Separating gel was cast as per the the composition. Ammonium Per Sulphate (APS) and TEMED were added just prior to pouring the gel.The solution was poured and allowed to polymerize between two glass plates. The gel was overlaid carefully with a film of distilled water. After polymerization, the water layer was removed and stacking gel was poured.The comb was placed on the top. The comb was carefully removed from the wells after polymerization of the stacking gel.The wells are washed with tank buffer. Equal volume of sample and 2X sample buffer was mixed and heat 10 to 15 min at 90^0c.These samples are kept in ice up to sample loading.20µl of the samples were then carefully loaded into the wells.125ml of electrode buffer was poured in the inner chamber and 200ml of buffer in the outer chamber. Gels were run at a constant current 100voltage for 90min (or) till the dye front reached the bottom of the gel. The gel was removed carefully from the plates and was placed in staining. Solution coo massive brilliant blue overnight. The gel was replaced into the destaining solution for one day and washed and it was expressed as kilo Dalton.

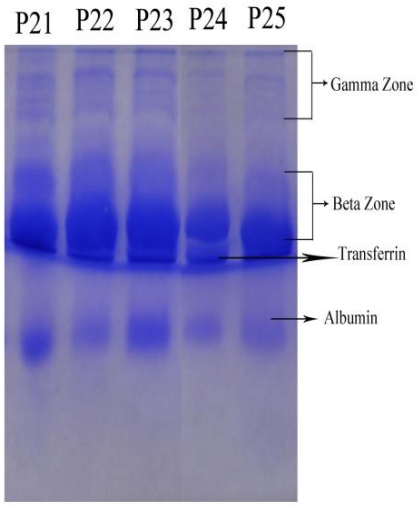
RESULT

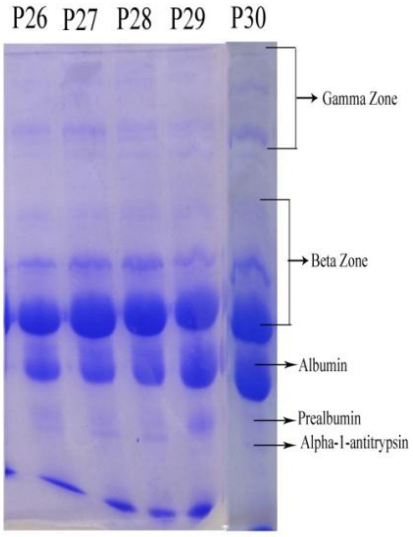

PRE ART
P1 P2 P3 P4 P5
Gamma Zone
Beta Zone
Albumin
Prealbumin
Alpha-1-antitrypsin

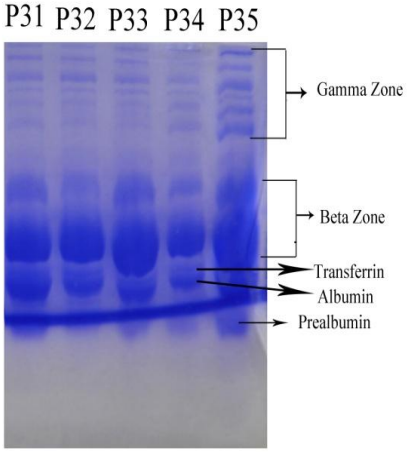

P6 P7 P8 P9 P10
Gamma Zone
Beta Zone
Transferrin
Albumin
Prealbumin
Alpha-1-antitrypsin

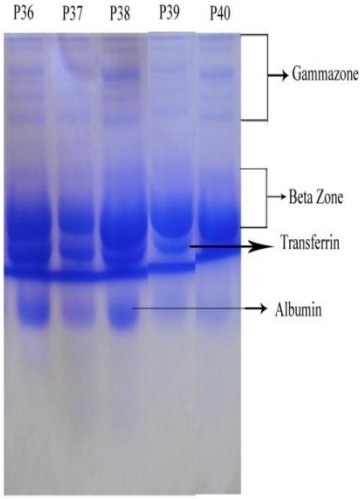

P11 P12 P13 P14 P15
Gamma Zone
Beta Zone
Albumin
Prealbumin
Alpha-1-antitrypsin

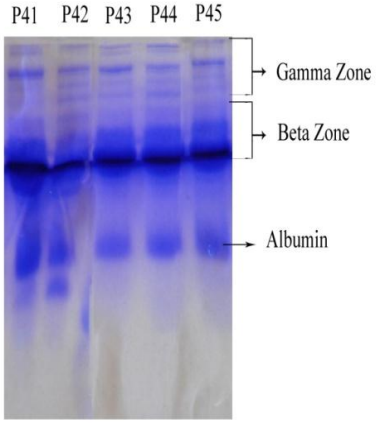

P16 P17 P18 P19 P20
Gamma Zone
Beta Zone
Albumin
Prealbumin
Alpha-1-antitrypsin

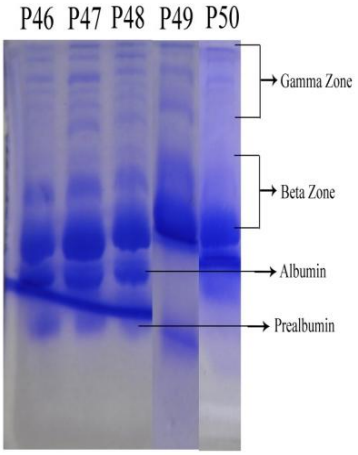

P21 P22 P23 P24 P25
Gamma Zone
Beta Zone
Transferrin
Albumin

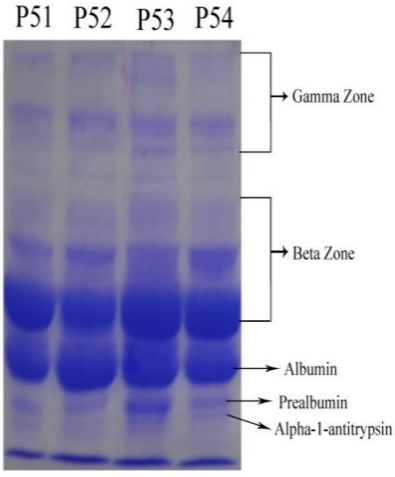

P26 P27 P28 P29 P30
Gamma Zone
Beta Zone
Albumin
Prealbumin
Alpha-1-antitrypsin

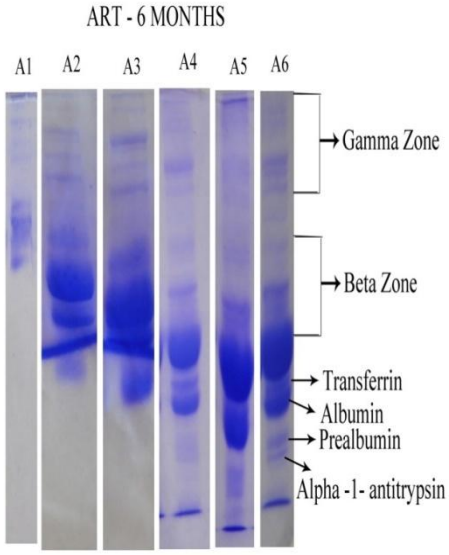

P31 P32 P33 P34 P35
Gamma Zone
Beta Zone
Transferrin
Albumin
Prealbumin

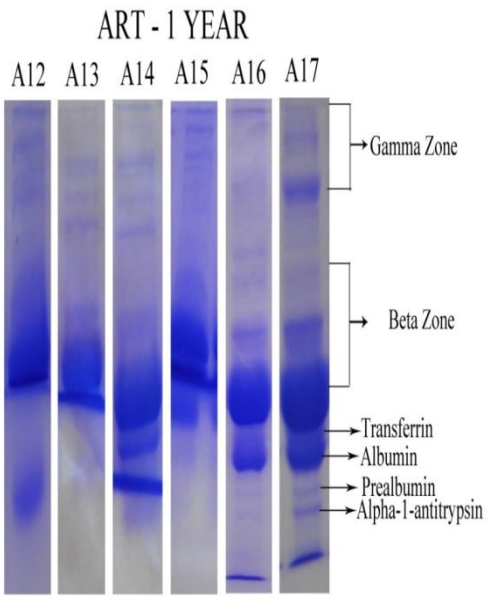

P36 P37 P38 P39 P40
Gammazone
Beta Zone
Transferrin
Albumin

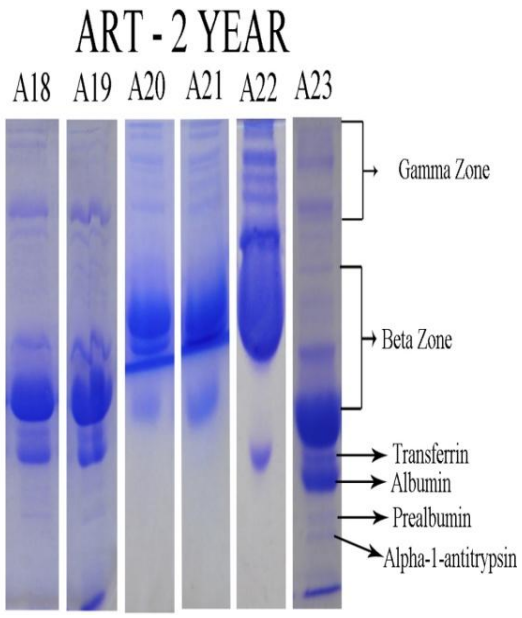

P41 P42 P43 P44 P45
Gamma Zone
Beta Zone
Albumin

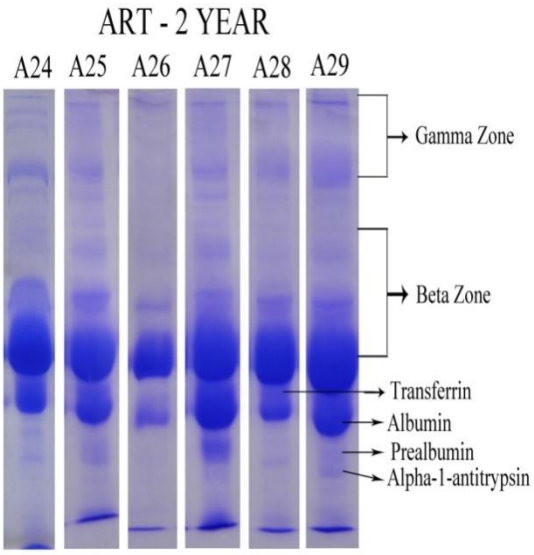

P46 P47 P48 P49 P50
Gamma Zone
Beta Zone
Albumin
Prealbumin

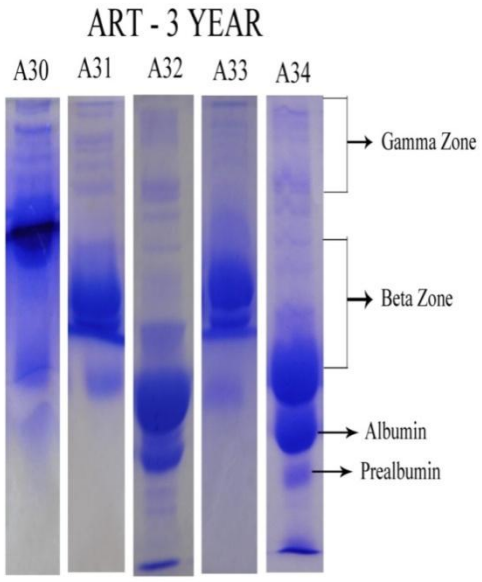

P51 P52 P53 P54
Gamma Zone
Beta Zone
Albumin
Prealbumin
Alpha-1-antitrypsin

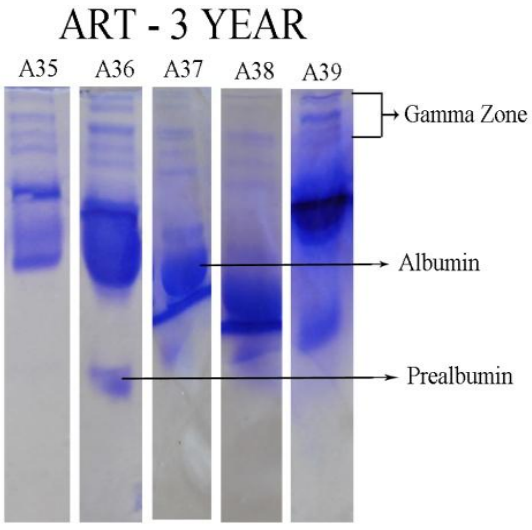

ART - 6 MONTHS
A1 A2 A3 A4 A5 A6
Gamma Zone
Beta Zone
Transferrin
Albumin
Prealbumin
Alpha -1- antitrypsin


ART - 1 YEAR
A12 A13 A14 A15 A16 A17
Gamma Zone
Beta Zone
Transferrin
Albumin
Prealbumin
Alpha-1-antitrypsin


ART - 2 YEAR
A18 A19 A20 A21 A22 A23
Gamma Zone
Beta Zone
Transferrin
Albumin
Prealbumin
Alpha-1-antitrypsin


ART - 2 YEAR
A24 A25 A26 A27 A28 A29
Gamma Zone
Beta Zone
Transferrin
Albumin
Prealbumin
Alpha-1-antitrypsin


ART - 3 YEAR
A30 A31 A32 A33 A34
Gamma Zone
Beta Zone
Albumin
Prealbumin


ART - 3 YEAR
A35 A36 A37 A38 A39
Gamma Zone
Albumin
Prealbumin

CD4 Enumeration of pre ART patients

S.No	Reference No	Patient ID No	Age & Sex	CD4 count
1	P1	3377	25/F	31
2	P2	3371	43/M	522
3	P3	3367	34/M	105
4	P4	3366	23/F	354
5	P5	3365	30/F	710
6	P6	3361	22/F	407
7	P7	3359	42/M	1
8	P8	3357	27/F	196
9	P9	3356	21/F	587
10	P10	3355	22/F	596
11	P11	3354	32/M	112
12	P12	3353	45/M	303
13	P13	3352	37/F	815
14	P14	3351	45/M	219
15	P15	3350	36/F	198
16	P16	3342	40/F	454
17	P17	3335	12/M	455
18	P18	3635	45/F	436
19	P19	3355	23/F	596
20	P20	3334	45/F	336
21	P21	3637	37/F	595
22	P22	3642	40/M	448
23	P23	3643	24/F	399
24	P24	3647	52/F	484
25	P25	3648	46/M	374
26	P26	3349	20/M	388
27	P27	3616	25/F	343
28	P28	3633	40/M	711
29	P29	3614	41/M	205
30	P30	3348	29/F	395
31	P31	3607	32/F	366
32	P32	3605	40/F	360
33	P33	3604	30/F	289
34	P34	3603	23/F	312
35	P35	3601	28/F	211
36	P36	3344	32/M	484
37	P37	3341	25/F	440
38	P38	3340	40/M	513
39	P39	3339	41/M	503
40	P40	3332	23/F	414
41	P41	3650	36/F	728
42	P42	3370	35/F	900
43	P43	3375	27/F	388
44	P44	3379	30/M	651

45	P45	3378	24/F	632
46	P46	3613	65/M	254
47	P47	3611	33/M	114
48	P48	3610	27/M	126
49	P49	3636	26/F	235
50	P50	3622	30/M	172
51	P51	3634	40/F	809
52	P52	3329	32/F	175
53	P53	3374	47/M	67
54	P54	3373	50/M	211

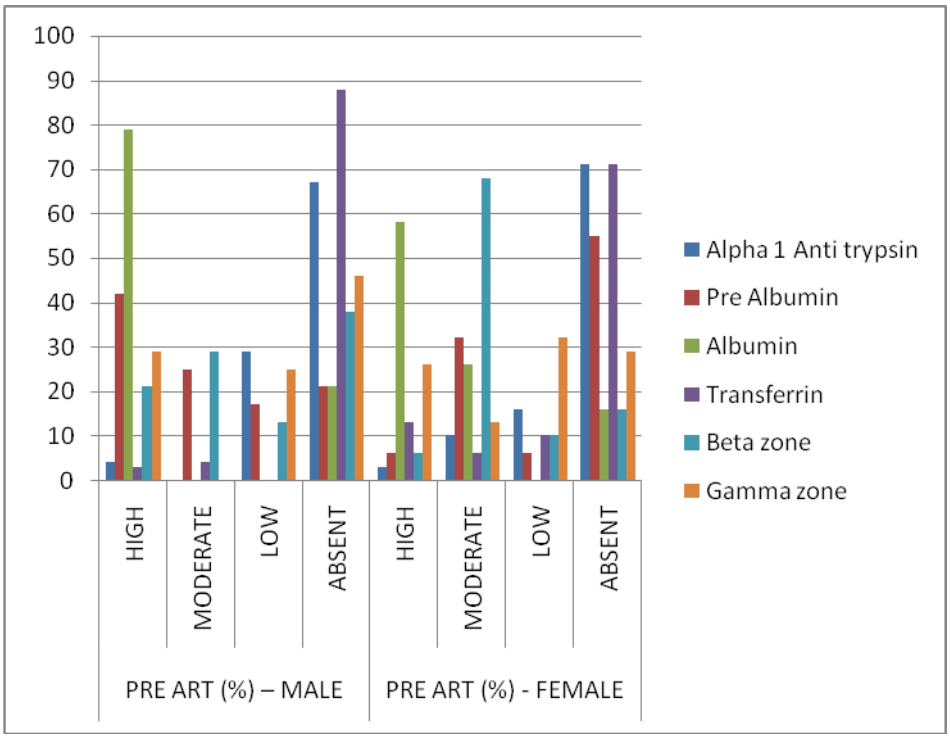
CD4 Enumeration of ART Patients

S.NO	REFERENCE NO	PATIENT ID NO	AGE&SEX	CD4 COUNT
1	A1	3362	32/F	394
2	A2	3608	62/F	132
3	A3	3612	17/M	374
4	A4	3363	47/F	400
5	A5	3330	32/F	274
6	A6	3343	60/F	135
7	A7	3336	43/M	408
8	A8	3347	65/M	334
9	A9	3358	38/M	315
10	A10	3360	58/M	211
11	A11	3621	26/F	241
12	A12	3649	47/M	176
13	A13	3615	30/F	569
14	A14	3609	25/F	268
15	A15	3620	30/M	222
16	A16	3619	33/M	188
17	A17	3364	20/F	689
18	A18	3380	24/F	535
19	A19	3328	3/F ch	1397
20	A20	3338	67/M	220
21	A21	3331	4/M ch	535
22	A22	3641	3/F ch	200
23	A23	3638	30/M	575
24	A24	3333	65/F	145
25	A25	3645	38/M	945
26	A26	3639	35/M	630
27	A27	3644	45/M	312
28	A28	3632	49/M	261
29	A29	3640	14/M	570
30	A30	3376	39/M	205
31	A31	3345	42/F	603
32	A32	3618	39/M	638
33	A33	3646	50/M	536
34	A34	3631	50/M	466

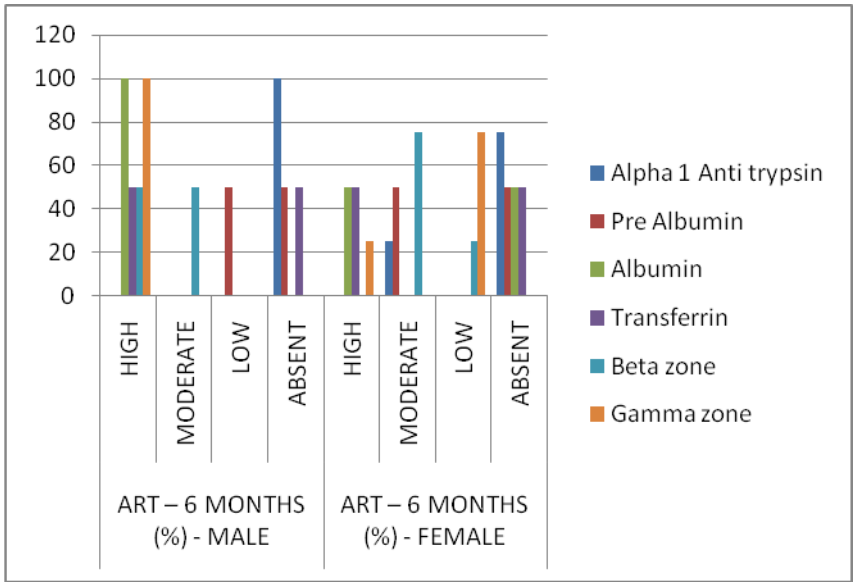
35	A35	3337	32/F	665
36	A36	3368	46/M	1084
37	A37	3606	47/M	99
38	A38	3617	42/M	63
39	A39	3372	35/M	292

Comparative values of protein level in pre ART Male and Female Samples

Name of bands /level	**ART 3 YEAR (%) – MALE**				**ART 3 YEAR (%) - FEMALE**			
	High	**Moderate**	**Low**	**Absent**	**High**	**Moderate**	**Low**	**Absent**
Alpha 1 Anti trypsin	0	11	0	8	0	0	0	100
Pre Albumin	33	11	0	5	0	0	0	100
Albumin	78	0	0	2	0	0	100	0
Transferrin	11	0	0	8	0	0	0	100
Beta zone	3	3	56	0	1	100	0	0
Gamma zone	56	11	22	1	100	0	0	0

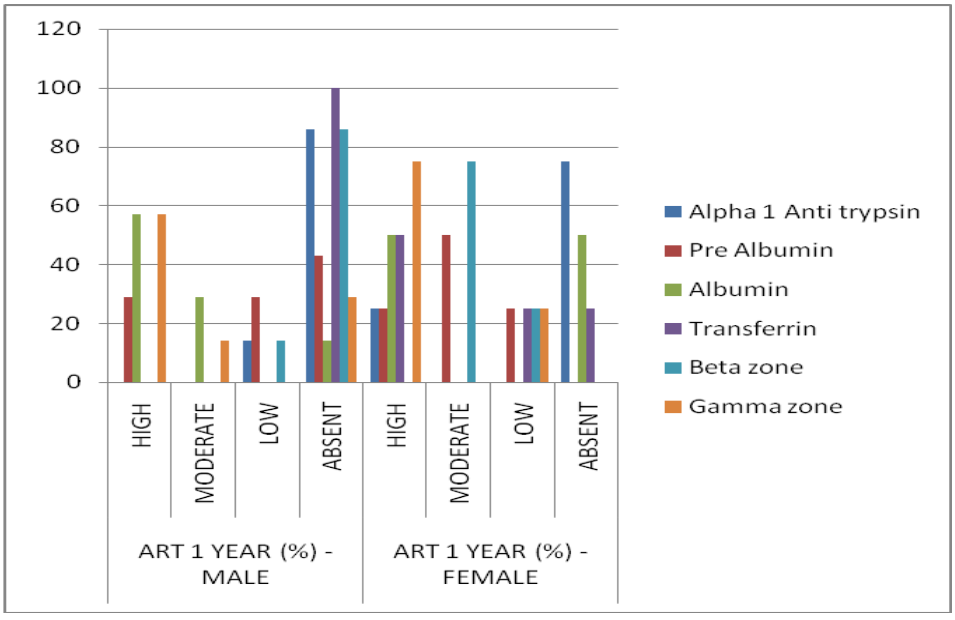


Comparative values of protein level in ART 6 months Male and Female samples

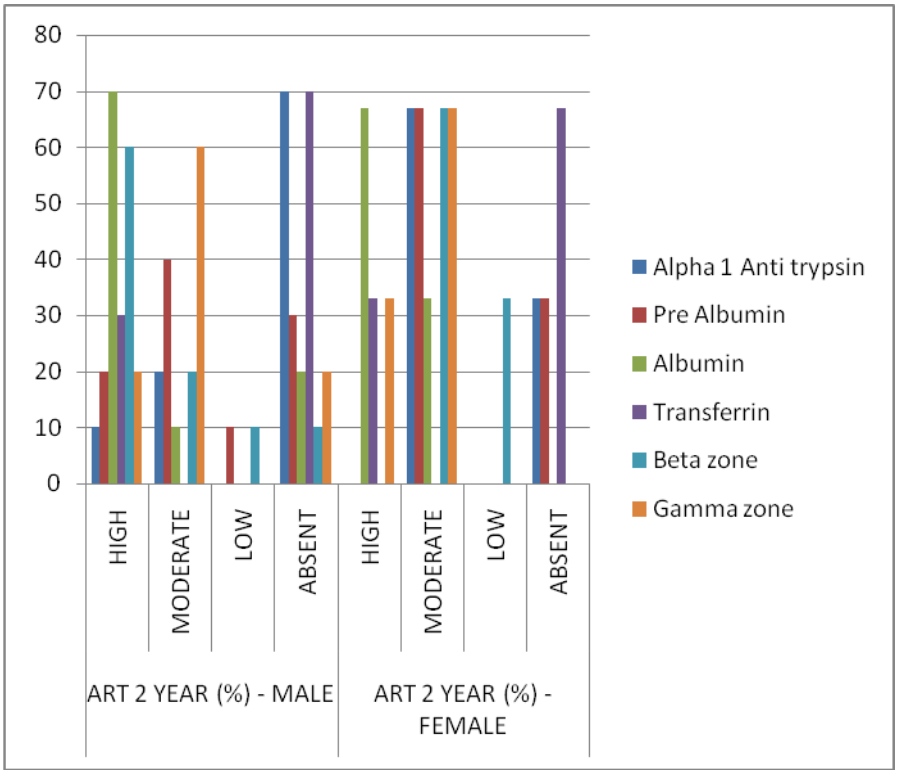


Comparative values of protein level in ART 1 year Male and Female

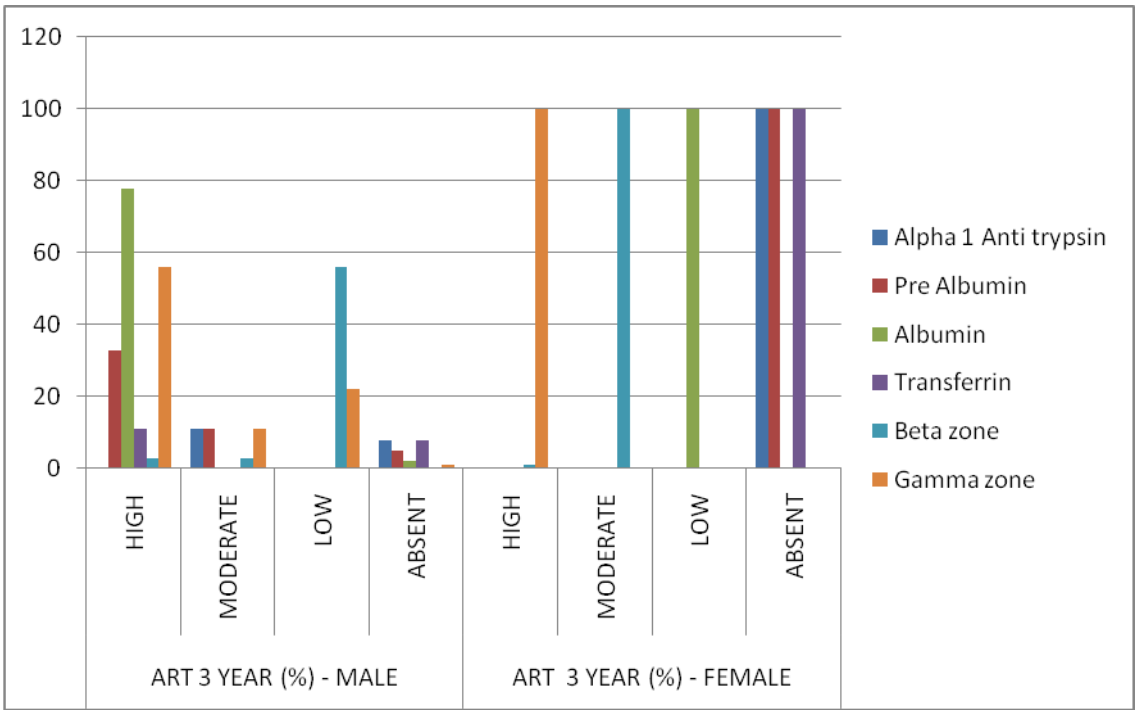
Name of bands /level	ART 2 YEAR (%) – MALE				ART 2 YEAR (%) - FEMALE			
	High	Moderate	Low	Absent	High	Moderate	Low	Absent
Alpha 1 Anti trypsin	10	20	0	70	0	67	0	33
Pre Albumin	20	40	10	30	0	67	0	33
Albumin	70	10	0	20	67	33	0	0
Transferrin	30	0	0	70	33	0	0	67
Beta zone	60	20	10	10	0	67	33	0
Gamma zone	20	60	0	20	33	67	0	0



Comparative values of protein level in ART 2 year Male and Female samples



Comparative values of protein level in 3 year ART Male and Female samples



Comparative values of protein level in pre ART Male and Female samples

PRE ART

Maximum number of pre ART female samples containing alpha 1 antitrypsin, albumin, gamma zone than male samples. Maximum number of male samples containing pre albumin.

ART-6 MONTHS

Maximum number of ART-6 months treated female samples containing alpha 1 antitrypsin, pre albumin, transferrin , beta zone, gamma zone. Maximum number of male samples containing albumin.

ART-1 YEAR

Maximum number of ART -1 year treated female samples containing transferrin and beta zone. Maximum number of male samples containing alpha 1 antitrypsin, pre albumin, albumin, gamma zone.

ART-2 YEAR

Male samples only containing all the proteins in maximum numbers.

ART-3 YEAR

Male samples only containing all the proteins in maximum numbers.

DISSCUSION

Alpha 1 antitrypsin

Alpha 1 antitrypsin deficiency is an inherited disorder that can cause lung disease in adults and liver disease in adults and children. Not having enough alpha 1 antitrypsin puts risk of emphysema or liver problems.Alpha-1 antitrypsin may play a protective role in HIV-1 infection (Potthoff *et al.*, 2007).

TRANSTHYRETIN or PREALBUMIN

In medicine, nutritional status can be assessed by measuring concentrations of transthyretin in the blood.In adults, prealbumin screening has been useful in assessing protein status in AIDS - or cancer-related cachexia, as well as nutritional status in nursing home residents.Higher prealbumin concentrations were associated with vascular access–related hospitalization.

ALBUMIN

Albumin is essential for maintaining the osmotic pressure needed for proper distribution of body fluids between intravascular compartments and body tissues.levels of serum albumin were only moderately related to traditional markers of HIV disease progression, including CD4 cell count, viral load and body mass index.The investigators note that "even though serum albumin is not a specific marker of HIV-1 infection, it is one of the strongest independent predictors of mortality."In women with severe immune damage, and a CD4 cell count below 200cells/mm^3, the risk of death was increased eight-fold in those with serum albuminbelow 35 mg/L compared to those with a level above 42 mg/L.

TRANSFERRIN

Transferrin is also associated with the innate immune system. **Transferrin** is a blood plasma protein for iron ion delivery .A patient with an increased serum transferrin level often suffers from iron deficiencyanemia. (*Macedo MF*, *de Sousa M* (March 2008).An anemia in the heart and liver that leads to many complications including heart failure.

GAMMA ZONE

IgG, IgA, IgD, IgE, IgM .Decreased or absent IgA termed selective IgA defiency,can be clinically significant immunodefiency. Neisseria gonorrhoeae destroys IgA.IgM antibodies appear early in the course of an infection.**Iron deficiency i**n populations with high levels of infection and HIV, an HIV test is necessary to interpret any tests of iron deficiency.

Immunoglobulin

low serum transferrin concentration in children with the nephrotic syndrome is related to their decreased immunoglobulin concentrations.Decreased serum transferrin concentrations might limit immunoglobulin synthesis.

IgA

The frequency is increased in children with recurrent respiratory tract infections especially in relatives patients with immunoglobulin deficiencies. (J. Litzman *et al*., (2000)).

IgE

Elevated serum immunoglobulin E (IgE) and increased prevalence of atopy is reported in patients infected with human immunodeficiency virus (HIV). In advanced stages of HIV, elevated serum IgE may be specific for antigens other than those known as allergens. (Corinna S *et al.* ,(2006)).

CONCLUSION

HIV patients are susceptible to opportunistic infections due to the deficiency of proteins.ART improves the CD4 count and proteins levels. 3 year after the treatment of ART, protein levels are increased in the male samples.

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