Western blot pattern in HIV positive individuals in Namakkal, South India.

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Citation

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Abstract

Objective: The objective of this study was to determine the western blot pattern among HIV positive individuals in different stages of HIV infection in Namakkal, South India.

Material and methods: After an informed consent, Western Blot test from 40 HIV seropositive patients attending the ART center at Namakkal, were performed using HIVBLOT 2.2, manufactured by Genelabs diagnostics, Singapore, as per the kit instructions. Demographic, clinical and diagnostic data were collected.

Results: The age ranged from 22-51 years (mean- 36 years). 47.5% were males and 52.5% were females. The patients were classified according to the WHO stages. Eleven patients were in Stage I, 10 each in Stage II and III and 9 patients were in Stage IV. With the exception of p24, the other GAG proteins, p55, p39 and p17 are not expressed very efficiently in Stage I patients. The expression of gp160, gp41, p66, p31 have all increased by approximately 10-20% in Stage II. In stage III, the ENV proteins gp160 and gp120 have been found in 90% of the cases. There was a 20% decrease in the incidence of p24 protein. Of the nine stage IV samples, 89% conform to the WHO interpretation criteria.

Conclusion: Interpretation of the WB band pattern in combination with clinical features may be occasionally useful in predicting the stage of HIV infection. This study suggests that antibodies to gag antigen P17 may be used as a marker for disease progression.

BACKGROUND

At present, the enzyme linked immuno sorbet assay (ELISA) is the most widely used serological test for the detection of antibodies to HIV [1, 2]. For diagnosis of clinically suspected cases and for voluntary testing, testing is performed with ERS (ELISA/Rapid/Simple) using HIV kits with different antigens in the government settings.[3]

Western blot assay is often regarded as the gold standard for confirmation of HIV serostatus. Although the overall sensitivity and specificity of the WB for detection of antibodies to the various viral proteins is high, there have been substantial differences in the timing of the appearance of antibody bands and their intensities during different stages of HIV infection[4]. The objective of this study was to determine the western blot pattern among HIV positive individuals in different stages of HIV infection in Namakkal, South India.

MATERIAL AND METHODS

After obtaining informed consent, blood was collected from 40 known HIV seropositive patients attending the ART center at Namakkal. 2ml of whole blood was collected in EDTA vaccutainer tube. The serum was separated from the whole blood. The samples were stored and transported in cold chain to the Department of Experimental Medicine and AIDS Research Center at the TN Dr. MGR Medical University in Chennai. They were stored at -20°C till further analysis in the laboratory. Western Blot test was performed using HIVBLOT 2.2, manufactured by Genelabs diagnostics, Singapore. The samples were processed as per the kit instructions.

Performa was filled to obtain demographic, clinical and diagnostic data. For the interpretation of the western blot strips, WHO criteria were followed.

RESULTS

The age of the patients ranged from 22-51 years with a mean

of 36 years. 47.5% were males and 52.5% were females. Average income of the patients were Rs.. 1660/month ($40/month). Majority (47.5%) were daily wage workers or otherwise called coolies

The patients were classified according to the WHO stages. Eleven patients were in Stage I, 10 each in Stage II and III and 9 patients were in Stage IV (figure 1).

Figure 1

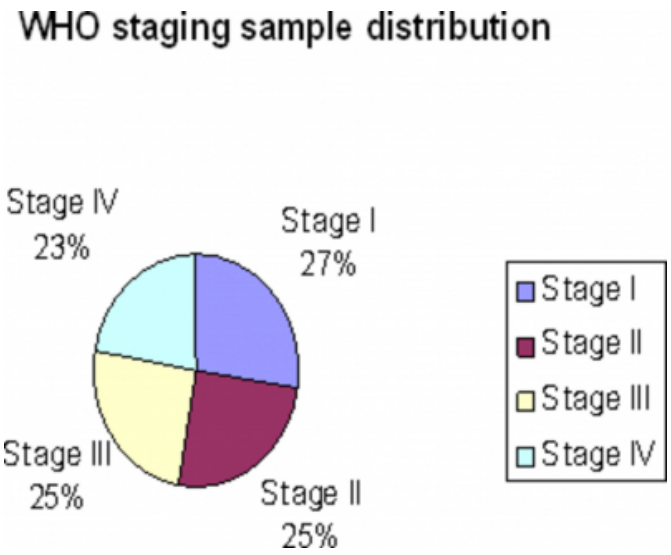

Figure 2

Stage I

BANDS	32	35	1	14	2	16	10	12	3	4	18	Mean
gp160		gp160		gp160	gp160	gp160	gp160	gp160	gp160	gp160	gp160	81.80%
gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	100%
p66		p66	p66	p66	p66	p66	p66		p66	p66	p66	81.81%
p55												0%
p51		p51	p51	p51	p51	p51	p51		p51	p51	p51	81.81%
gp41	gp41	gp41		gp41	gp41	gp41	gp41	gp41	gp41	gp41	gp41	90.90%
p39		p39		p39	p39		p39				p39	45.45%
p31	p31	p31		p31	p31	p31	p31	p31	p31	p31	p31	90.90%
p24	p24	p24	p24	p24	p24	p24	p24	p24	p24	p24	p24	100%
p17	p17	p17	p17	p17	p17		p17				p17	63.63%
Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	100%
HIV-2												

The gp120 and p24 protein bands are present in all the samples. The gp160, p66, p51 bands are present in 82% of the samples. p31 and gp41 bands are present in 91% of the patients The p55 band is absent in all the samples. The p39 band is present in 45% of the samples and the p17 band is present in about 64% of the samples. From these results we see that with the exception of p24, the other GAG proteins, p55, p39 and p17 are not expressed very efficiently in Stage I patients.

Figure 3

Stage II

BANDS	26	12	17	9	15	4	5	6	2	13	Mean
gp160	gp160	gp160	gp160	gp160	gp160	gp160	gp160	gp160	gp160	gp160	100%
gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	100%
p66	p66	p66	p66	p66	p66	p66	p66	p66		p66	90%
p55											0%
p51	p51	p51	p51	p51	p51		p51	p51		p51	80%
gp41	gp41	gp41	gp41	gp41	gp41	gp41	gp41	gp41	gp41	gp41	100%
p39			p39	p39			p39	p39			40%
p31	p31	p31	p31	p31	p31	p31	p31	p31	p31	p31	100%
p24	p24	p24	p24	p24	p24	p24	p24	p24			80%
p17	p17	p17	p17			p17		p17			50%
Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	100%
HIV-2											

The ENV proteins, gp160, gp120, gp41 and the POL protein, p31 are present in all the samples. Contrastingly, p55 is absent in all the samples. p39 is present in 40% of the samples, while p17 is present in 50% of the samples. p24 and p51 bands are present in 80% of the samples, while p66 is present in 90% of them. Here, we observe that the GAG proteins, p39 and p17 are expressed in less number of patients. And the other GAG protein, p55 is practically non-existent in these patients. Though p24 has been expressed in a majority of the cases, there has been a 20% decrease in its expression on comparison between the stage I and stage II samples. There is a marginal decrease of 5% in the expression of p39, while, a decrease of 14% is observed in the case of p17 on comparison of the Stage I and stage II samples. The expression of gp160, gp41, p66, p31 have all increased by approximately 10-20%. However, the expression of p51 appears to be the same in both the cases. The trend of absence of bands has been observed more in the GAG proteins as in the stage I samples.

Figure 4

Stage III

BANDS	30	33	8	13	18	7	11	8	7	5	Mean
gp160	gp160	gp160	gp160		gp160	gp160	gp160	gp160	gp160	gp160	90%
gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	100%
p66	p66	p66	p66	p66	p66	p66	p66	p66	p66	p66	100%
p55			p55	p55	p55						30%
p51		p51	p51	p51	p51	p51	p51	p51		p51	80%
gp41	gp41	gp41	gp41		gp41	gp41	gp41	gp41	gp41	gp41	90%
p39			p39	p39	p39						30%
p31	p31	p31	p31	p31	p31	p31	p31	p31	p31	p31	100%
p24	p24		p24	p24	p24			p24	p24		60%
p17			p17	p17	p17						30%
Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	100%
HIV-2											

The ENV proteins gp160 and gp120 have been found in 90% of the cases. gp120, p66 and p31 have been found in all the samples. p55, p39 and p17 are present in 30% of the samples. p51 was observed in 80% of the samples, while, p24was found in 60% of them. On comparison with the stage II samples, it was found in the stage III samples that there was a 20% decrease in the incidence of p24 protein. The expression of p39 and p17 has been reduced to 30%. But, surprisingly, there is a 30% increase in expression of

p55. There is decrease of 10% in the expression of gp160 and gp41, while there is an increase of 10% in the expression of p66 on comparison of stage II and stage III samples. The decrease in appearance of bands is again seen to be confined to the GAG proteins. While, there is a consistent decrease in the p24 band from stage I to stage III, the decrease in the other GAG bands seems to be inconsistent, though there is a considerable decrease in the expression levels. The decrease or increase in the appearance of the other proteins is inconsistent from stage I to stage III.

Figure 5

Stage IV

BANDS	27	22	24	11	3	6	14	16	1	Mean%
gp160	gp160	gp160		gp160	gp160	gp160	gp160	gp160	gp160	88.88%
gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	gp120	100%
p66	p66	p66	p66	p66	p66	p66	p66	p66	p66	100%
p55	p55		P55		P55					33.33%
p51	p51	p51	p51	p51	p51	p51	p51	p51	p51	100%
gp41	gp41	gp41		gp41	gp41	gp41	gp41	gp41	gp41	88.88%
p39	p39		p39		p39				p39	44.44%
p31	p31	p31	p31	p31	p31	p31	p31	p31	p31	100%
p24	p24	p24	p24	p24	p24	p24	p24	p24	p24	100%
p17	p17		p17		p17					33.33%
Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	100%
HIV-2										

Of the nine stage IV samples, 89% conform to the WHO interpretation criteria. The gp160 and gp41 bands are present in 89% of the samples. gp120, p66, p51, p31 and p24 proteins are present in all the samples. p55 and p17 are present in 33% of the samples. p39 is present in 44% of the samples. The expression of p39 has increased by about 14%. p17 and p55 expression is almost constant. There is an increase of expression in p24 and p51. The trends in the ENV and POL proteins do not indicate any changes in band profiles according to the stage. But the GAG proteins show differences in the various WHO stages of HIV infection. However, the fourth stage band patterns do not conform to the trend. The other ENV and POL proteins do not show any trend whatsoever.

Figure 6

Cumulative means of band appearance in various stages

BANDS	Stage I	Stage II	Stage III	Stage IV
gp160	81.80%	100%	90%	88.88%
gp120	100%	100%	100%	100%
p66	81.81%	90%	100%	100%
p55	0%	0%	30%	33.33%
p51	81.81%	80%	80%	100%
gp41	90.90%	100%	90%	88.88%
p39	45.45%	40%	30%	44.44%
p31	90.90%	100%	100%	100%
p24	100%	80%	60%	100%
p17	63.63%	50%	30%	33.33%
Control	100%	100%	100%	100%
HIV-2				

DISCUSSION

In this study it was observed that antibodies to the envelope (ENV) precursor protein gp160 and the final ENV proteins (gp120 and gp41) can be detected in specimens from majority of the HIV-infected persons regardless of clinical stage. This finding is in agreement with other studies[5,6] The gp41 reactive band was seen in most of the patients however it was slightly less seen in patients with advanced disease progression as in stage IV. Antibodies to polymerase gene products (pol)- p66, 51 and 31 were conserved well in all stages of HIV disease. Similar observations have been reported earlier [12].

Antibodies against p24 was detectable in early and as well as late stage HIV disease. This is unlike the studies reported from Europe and North America [7, 8] that describe that loss of P24 antibody is a marker for advanced HIV-1 infection. However studies from Africa and India [6, 9] has similar observations as we have observed in our study on the persistence of p24 antibodies in late disease progression. Studies are needed to find out if this has anything to do with HIV subtypes or due to factors other than HIV infection.

On the other hand, this study has noted gradual loss of reactivity to P17 antigen from Stage I to Stage IV of the disease. Antibodies against p17 was detected in 64% of Stage I patients but only in 33% of Stage IV patients. Similar observations has been reported which suggest that decline of antibody reactivity to p17 may be an earlier serological marker for disease progression in HIV infection [10, 11]. In this study p55 reactive band was undetectable in patients with stage I and II and 30%-33% detectable in patients with stage III and IV respectively. This is unlike a

study from India which reported gradual absence or presence of faint band to p55 with clinical progression of the disease [6].

CONCLUSION

Interpretation of the WB band pattern in combination with clinical features may be occasionally useful in predicting the stage of HIV infection. This study also suggests the antibodies to gag antigen P17 may be used as a marker for disease progression.

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