Original Article

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Interferon Gamma and Soluble MICA as A New Biomarker for Immunological Severity Detection in HIV Infected Patients Undergoing Retroviral Therapy

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Abstract:

Objective: Variations in immune responses to viral particles and antiretroviral drugs show interference of the cluster of differentiation number 4 T cell monitoring methods, leading to an inaccurate evaluation for human immunodeficiency virus infected patients receiving antiretroviral therapy. Other biological markers; such as cytokine; especially interferon gamma (IFN-γ) and soluble molecules of major histocompatibility complex class I chain related molecule A (sMICA), were designed as a novel severity marker.

Material and Methods: Levels of IFN-γ and sMICA were evaluated for 69 patients, who presented with HIV infection; with no other diseases or inflammation. The candidates were classified into four groups, depending on their CD4 T cell count. The IFN-γ and sMICA serum content of the patients was detected in triplicate, using enzyme-linked immunosorbent assay.

Results: Mean value of IFN-γ in each severity group was 49.1±9.7, 69.1±21.8, 63.0±12.8, and 69.4±18.4 picograms/milliliter for none, mild, advanced, and severe cases, respectively. sMICA was detected at 36.8±19.6, 134.7±122.5, 33.6±12.4, and 83.9±40.0 international unit/milliliter, respectively. A significant association between IFN-γ and CD4 cells of normal anti-retroviral treatment response, defined by CD4 cells and viral loads, was observed using Spearman correlation, with p-values 0.033, r -0.434, 0.026 and -0.321, respectively.

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Conclusion: IFN-γ and sMICA were found to be associated with the CD4 count in the normal responses to antiretroviral treatment. This suggested that IFN-γ could be used as a biological marker for monitoring of immunological severity during anti-retroviral responses.

Keywords: CD4 cells, HIV, IFN-γ, NK cells, sMICA

Introduction

Human immune deficiency virus (HIV) infection is a major problem in Thailand, and while the government has promoted a strategy to ameliorate this disease, new cases are still reported every year. The cluster of differentiation number 4 (CD4) count is currently used as a monitoring biomarker for HIV infected patients to evaluate severity levels.[1] However, monitoring is expensive, with each patient being monitored twice a year, following the Thailand monitoring strategy. Patients diagnosed with HIV infection are promptly treated with antiretroviral drugs.[2] These drugs are composed of both nucleoside and protein inhibitors, which affect viral replication with other protein functions.[3] Previous evidence suggested that patients undergoing antiretroviral therapy had CD4 cell counts from 164 to 1,286 cells/cubic millimeter, with a similar viral load in blood samples.[4] The use of CD4 count for monitoring treatment in these groups of patients must be performed with care. So, other biological markers that occur during immune response; such as cytokines; especially interferon gamma (IFN-γ), were designed as a new severity marker, which could indicate a patients' immune status and function in clearing infection during treatment.

IFN-γ is a major cytokine released during the immune response of T cells, and natural killer (NK) cells during viral infection.[5] Release of IFN-γ during HIV infection is the result of T cell activation, via HIV ribonucleic acid, by the Toll-like receptor 7 (TLR7). However, patients undergoing antiretroviral therapy (ART) show an abnormality with this mechanism. Prolonged ART treatment resulted in immune exhaustion and lower cytokine production; especially in IFN-γ, interleukin (IL)-10, IL-13, IL-17A, IL-5, and IL-6 in the undetectable viral load patient group.[6,7] Instead, Tat, Rev, Env, Gag, and Vif induced T cells releasing Granzyme B is helpful in protecting viral replication. This mechanism is mainly found in responses of another cell type named: NK cells.[8] However, as well as IFN-γ soluble molecules of major histocompatibility complex class I chain related molecule A (sMICA) also plays a crucial role in immune responses during HIV infection, and is involved in NK cell activation.[9] MICA is the ligand expressed on most viral infected cells or cancer cells.[10] This molecule is recognized by the NKG2D receptor, which is the co-stimulatory receptor expressed on cluster of differentiation number 8 positive (CD8+) T cells, and the most important activating receptor on NK cells.[11] MICA secreted into the blood circulation is called sMICA. This inhibits the function of CD8+ T cells and NK cells through the endocytosis of the NKG2D receptor. This mechanism triggers failure in immune cell response to infected cells.[12] In the early stage of HIV infection, there is some evidence suggesting an effect of this molecule in lowering of T and NK cell activation. It is suggested that sMICA plays a crucial role in controlling T and NK cells activation, which is a source of IFN-γ production and viral clearance.[13]

As previously mentioned, antiretroviral therapy drugs cause many manifestations in patients; such as, manipulating cytokine expression profiles in immune responses. This manifestation results in the release of matrix metalloproteinase enzyme, which is the cause

of sMICA shedding, and may lead to inhibition of NK and T cell activation as well as IFN-γ production.[14] This suggests that a substrate in the patients' serum; such as, IFN-γ and sMICA; which is released during HIV infection with antiretroviral therapy, may reflect a patient's immune response. Additionally, it could be used as a tool for prediction of immunological severity, instead of the CD4 cell count. This study evaluated the correlation of IFN-γ and sMICA, with four different immunological severities of HIV infections; defined by CD4 cell count as per the World Health Organization (WHO) criteria, in patients during antiretroviral therapy. The accuracy of using this new severity indicating marker for HIV treatment was assessed.

Material and Methods

This study estimated IFN-γ and sMICA levels in the serum of patients with HIV infection at different severities. Samples were collected from the HIV Care Clinic in Dok Khamtai Hospital during 2019. Sixty-nine study samples passed the following three inclusion criteria. Firstly, patients were fully investigated, diagnosed with HIV infection, by Thailand's national guidelines on HIV/AIDS treatment and prevention 2017[15], and were treated with antiretrovirals. Secondly, the severity of the samples was estimated consistently every six months by CD4 count detection. Thirdly, patients included in this study had no evidence or history of opportunistic infection, inflammation, or cancer, detected by their white blood cell count within the normal range of 5,000 to 10,000 cells/microliter.

The severity classification was performed following the immunological classification for HIV infection by the WHO. This staging criteria cloud classified patients into four different severity groups, depending on their CD4 count. The dour groups of immunological severity were composed of: none, mild, advanced, and severe, with the CD4 counts at >500, 350-499, 200-349, and <200 cells/microliter, respectively.[16] Then, the viral load and the decreasing rate of CD4 after treatment were used as an indicator of the patients' responses to ART treatment. The expected responses were defined by the viral load being below 20 copies/milliliter and the CD4 decreasing rate at 35 cells/microliter/year.[17]

In the first case, we used our previous data to estimate the sample size of each group from our study, from this data only 20 cases with severe form were found.[18] From the 20 samples in all severity groups, we were only able to collect nine severe cases; due to the high efficacy of antiretroviral drugs and the health care system.

The IFN-γ and sMICA in the patients' serum were measured using the ELISA technique. The standard method for detection of IFN-γ was performed as described in a handout from the BD optEIA Human IFN-γ ELISA kit. Similarly, the sMICA detection method also followed the protocol detailed in a handout from the R&D Duoset® Human MICA kit: all samples were tested randomly. Only twenty samples were tested at a time, using batch testing in parallel with one control sample: batch testing was conducted in triplicate. The results of each batch testing was accepted, and used for further analyses when the control sample was within an acceptable range of 10.0% compared to each testing. Levels of IFN-γ and sMICA were calculated from a standard curve, with a slope of more than 0.950 in each batch testing. Levels of IFN-γ and sMICA of the same sample from three batch testing results were averaged, and then used for further analyses.

Statistical analyses were performed using Statistical Package for the Social Sciences for Windows version 24 and GraphPad Prism 9 software (GraphPad, San Diego, CA). Different IFN-γ or sMICA levels were compared between diverse severity forms of illness in HIV infected patients. The Kruskal-Wallis one-way analysis was used for nonparametric testing, with 95.0% confidence. Correlations between the detection of IFN-γ and sMICA in the patients' serum with severity outcomes were also analyzed using

chi-square, while correlation of the IFN-γ sMICA and CD4 count in each severity was assessed using the non-parametric Spearman correlation test.

Results

Characteristics and immunological status of included samples

Sixty-nine HIV infected samples with different degrees of severity were included: comprising of 36 females and 33 males. Gender proportions were not significantly different, indicating that gender was not a factor affecting the results. All samples were in a healthy condition after receiving treatment with antiretroviral therapy. The samples did not present signs of opportunistic infection during the study, and the white blood cell counts of each patient were in the normal range (5,000-10,000 cells/cubic milliliter); additionally, the samples had no hospitalization records for any infection illness.

Samples were classified into four immunological severity groups as: none, mild, advanced, and severe, by the number of CD4 cells on the day that the samples were collected. The mean values of CD4 cells of each severity group were 923.9, 429.3, 281.1, and 119.3 cells/microliter, respectively (Table 1). No interception of CD4 count between each severity group was indicated via this classification method. Age is known to be an important factor affecting human immune responses and cytokine production. To remove this effect from our study, samples within different severities were compared with the patients age. The results demonstrated no significant difference between the patients age within each severity group, leading to the conclusion that age was not a factor involved in our study results. In addition, more than eighty percent of these patients responded well to ART treatment, which was indicated by the viral load being lower than 20 copies/milliliter (Table 1).

Table 1 Characteristics and Immunological status of inclusion samples

		SEX		Viral load		AGE	CD4	IFN-γ	sMICA
		Male Count (%)	Female Count (%)	<20 (copies/mL) Count (%)	>20 (copies/mL) Count (%)	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
Severity	None	12 (40.0)	18 (60.0)	25 (86.2)	4 (13.8)	46.7±1.3	923.9±67.8	49.1±9.7	36.8±19.6
	Mild	14 (77.8)	4 (22.2)	20 (95.2)	1 (4.8)	46.9±1.7	429.2±9.3	73.4±23.6	138.9±133.7
	Advanced	6 (40.0)	9 (60.0)	23 (88.5)	3 (11.5)	48.1±1.4	281.11±9.5	60.1±12.1	37.7±12.7
	Severe	4 (66.7)	2 (33.3)	12 (100)	0 (0.0)	48.7±1.9	119.3±13.3	69.4±18.4	83.9±40.0
IFN-γ	Not found	11 (50.0)	11 (50.0)	28 (90.3)	3 (9.7)	44.4±1.1	481.2±60.0	0.0±0.0	41.4±16.9
	Found	25 (53.2)	22 (46.8)	52 (91.2)	5 (8.8)	48.6±1.0	521.0±51.2	92.3±9.5	81.76±49.8
sMICA	Not found	25 (49.0)	26 (51.0)	55 (93.2)	4 (6.8)	47.5±0.9	553.8±52.2	60.8±10.2	0.0±0.0
	Found	11 (61.1)	7 (38.9)	25 (86.2)	4 (13.8)	46.4±1.4	413.3±51.4	61.0±11.6	206.1±97.1
ART responses	Normal	31 (50.8)	30 (49.2)	80 (100)	0 (0.0)	47.1±0.8	503.3±43.1	59.3±8.4	74.5±37.8
	Lower	3 (60.0)	2 (40.0)	0 (0.0)	8 (100)	47.4±1.7	570.4±121.9	77.8±27.7	16.9±10.5

IFN-γ=interferon gamma, CD4=cluster of differentiation number 4, sMICA=soluble major histocompatibility complex class I chain related molecule A, SEM=Standard Error of the Mean, ART=Anti-Retroviral Treatment

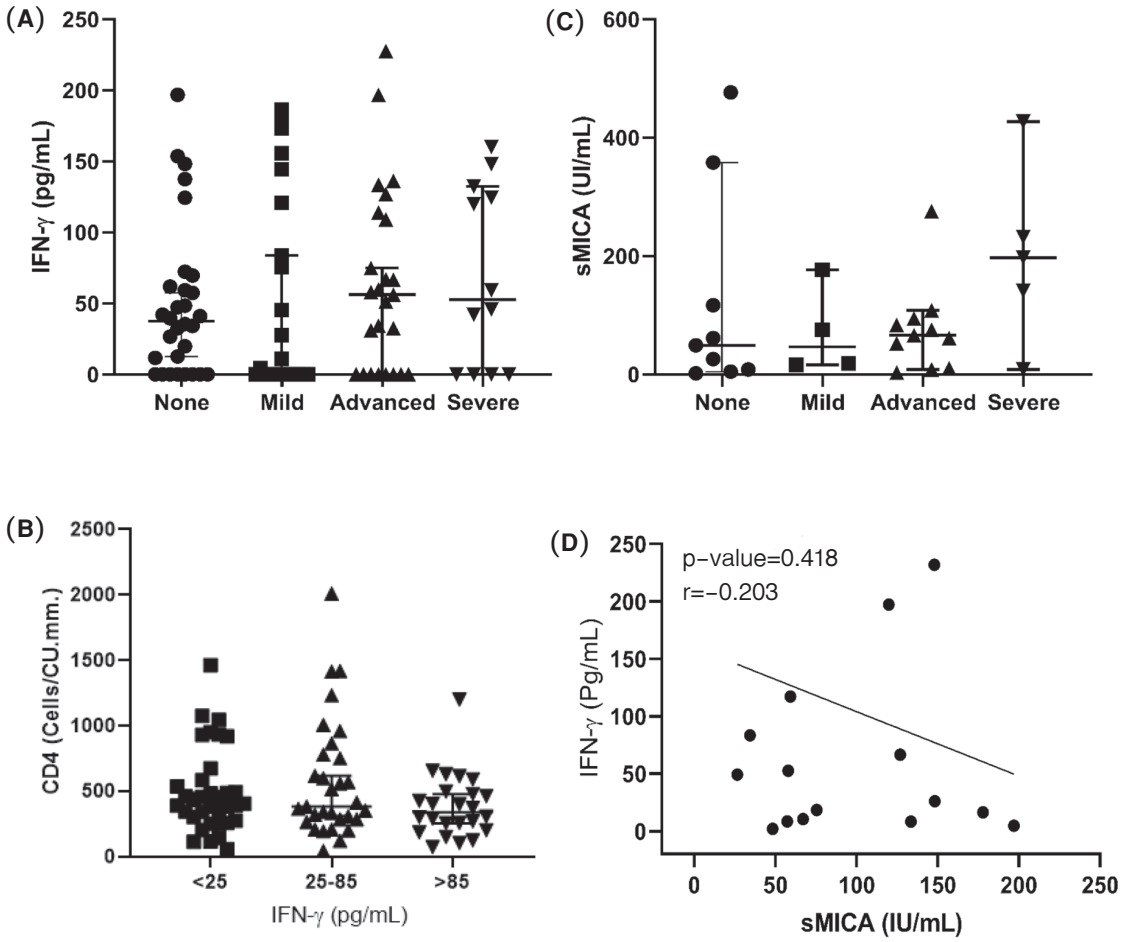
Level of IFN-γ and sMICA in serum of HIV infected patients and immunological severity

The level of serum cytokine, composed of IFN-γ and sMICA, during HIV infection with antiretroviral therapy was evaluated by triplicate testing, and the results demonstrated no significant difference between IFN-γ levels of each severity group (p-value=0.852). Median values of IFN-γ in none, mild, advanced, and severe groups were 49.1±9.7, 73.4±23.6, 60.1±12.1 and 69.4±18.4 picograms/milliliter, respectively (Figure 1A). An increasing trend of IFN-γ levels was shown in the more severe groups compared to the lower ones. However, upon further and more detailed examination of the graph, it was revealed that patients within each severity group could be separated into three different groups by their IFN-γ level. this, the patient's serum IFN-γ was used to divide the samples into three different groups as: low, moderate, and high IFN-γ, using IFN-γ at 25 and 85 picograms/milliliter as the cutoff point. The correlation between serum IFN-γ and CD4 cell count was investigated, and it demonstrated that there is no significant difference in the CD4 count between each group of patients with different serum IFN-γ levels (Figure 1B). These results suggested that some individual factors; such as, the genetical polymorphism within the patient; involving IL-4, IL-10 and IFN-γ itself, may cause different IFN-γ production and release in individuals.[20] We investigated whether this factor resulted from sMICA in these patients' serum, as one of the possible molecules in immune control: sMICA levels were estimated in the same manner as previously described. The results demonstrated that only 26.0% of the samples detected sMICA in serum, with a very low-level being present during HIV infection under antiretroviral treatment (Table 1). There was no significant difference when comparing sMICA levels between each severity group, with a p-value of 0.327. Median sMICA values in each group were 36.8±19.6, 138.9±133.7, 37.7±12.7 and 83.9±40.0 international unit/milliliter for none, mild, advanced, and severe groups, respectively (Figure 1C). Interestingly, we found an increase of serum sMICA in those with more severity; especially in the advanced group (Table 1), suggesting that the presence of sMICA may lead to low immune activation and virus control. The correlation of sMICA and serum IFN-γ were analyzed in nineteen patients, who presented with both sMICA and IFN-γ, and no significant correlation in a decreasing trend of IFN-γ was found in patients with higher serum sMICA; with a p-value of 0.413 and r=-0.203 (Figure 1D). This is suggestive of a very low effect of sMICA in the lowering of IFN-γ release during HIV infection.

Effect of serum IFN-γ and sMICA on the CD4 and Viral load in HIV patients via antiretroviral therapy

A trend of increasing IFN-γ and sMICA was detected at different HIV immunological severity levels. The correlation of IFN-γ and immunological severity levels was analyzed using chi-square. The results demonstrated that the presence of IFN-γ was not associated with any severity during HIV infection with antiretroviral therapy; as classified by CD4 number. However, the expected outcome of using anti-retroviral therapy is to reduce viral replication, and to increase CD4 cells. The data were then analyzed, as to whether these molecules were involved in the changes of the CD4 count during anti-retroviral therapy. Firstly, the samples were classified using the rate of CD4 decrease per year, so as to reflect the outcome of the therapy. Two groups of patient responses were classified as normal and lower responses, by the CD4 decreasing rate of <35 and >35 cells/microliter/year, respectively. Chi-square testing indicated that the presence of IFN-γ in the patients' serum had been found mainly in the normal response group, with a p-value of 0.006 and relative risk ratio at 0.584 compared to the lower response group, respectively (Table 2).

However, there was no correlation between the presence of serum sMICA and each immunological severity with chi-square, and p-value>0.050. When we reanalyzed this correlation within the CD4 decreasing rate, as previously described, sMICA was not significantly correlated with any responses with the p-value at 0.129; indicating that the presence of serum sMICA may be not involved in responses to antiretroviral therapy.



IFN-γ=interferon gamma, sMICA=soluble major histocompatibility complex class I chain related molecule A

Figure 1 (A and C) The level of IFN-γ and sMICA in patient serum, with the different severity levels, during HIV infection and anti-retroviral treatment. (B)The different CD 4 count in patients with three different groups of serum IFN-γ levels is not significantly different. (D) No significant correlation of serum IFN-γ and sMICA levels in patient's serum.

Table 2 Chi-square testing for interferon gamma and soluble major histocompatibility complex class I chain related molecule A on severity of human immune deficiency virus infection, based on cluster of differentiation number 4 decreasing rate and viral load

	IFN-γ		p-value	sMICA		p-value
	Not found	Found		Not found	Found	
CD4 decreasing						
Normal (<35 cell/microliter/year)	21	29	0.006*	30	20	0.129
Lower response (>35 cell/microliter/year)	0	12		10	2	
Total	21	41		40	22	
Viral load						
Normal (<20 copies/microliter)	18	36	0.552	36	18	0.438
Lower response (<20 copies/microliter)	3	5		4	4	
Total	21	41		40	22	

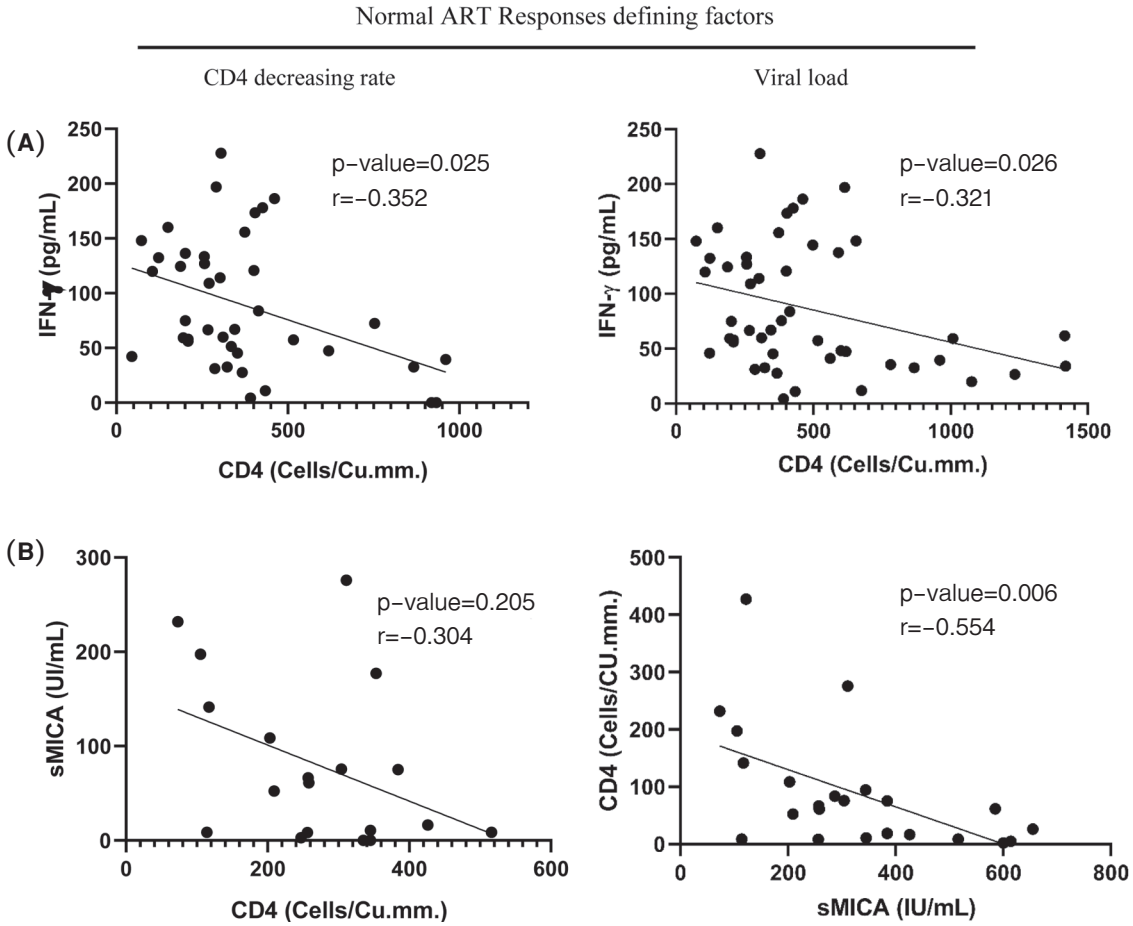
IFN-γ=interferon gamma, CD4=cluster of differentiation number 4, sMICA=soluble major histocompatibility complex class I chain related molecule A

Secondly, another factor indicating the success of anti-retroviral therapy was the blood viral load detection being used as an indicator. However, the detection of whether IFN-γ and sMICA in the patients' serum found was not significantly associated with the patients' responses to anti-retroviral treatment, as defined by the viral load. The p-value of chi-square testing for IFN-γ and sMICA on the patient's viral load responses were 0.552 and 0.438, respectively

IFN-γ and sMICA as markers for immunological prognosis

A low correlation was demonstrated between IFN-γ and sMICA, and immunological severity of HIV infection during antiretroviral therapy. After this, we investigated further to ascertain if these two molecules could be used as a novel marker for prognosis of immunological status in HIV infected patients. Correlation between IFN-γ or sMICA on the CD4 cells was studied, in patients with normal responses to anti-retroviral. Only patient's presenting with either of these molecules were included in this analysis. From this, only 40 and 19 cases were included in analyzes of IFN-γ and sMICA, respectively. The results demonstrated a significant association between IFN-γ and CD4 cells, by Spearman correlation, in patients with normal responses to anti-retroviral treatment in both categories manner. The correlation was shown in the group of CD4 decreasing rates and viral loads, with a p-value of 0.033 with r -0.434, and a p-value of 0.026 with r -0.321, respectively (Figure 2A). Similarly, sMICA showed a significant trend with the normal response group, defined by viral load, as found in IFN-γ (Figure 2B). The correlation between the sMICA and CD4 count was a p-value of 0.006 with r -0.554. This result suggested serum IFN-γ as a good candidate for a novel prognosis marker for HIV infected patients in a restricted group with normal responses to anti-retrovirus. This represented the highest population of patients undergoing antiretroviral therapy.

As previously mentioned, we found that the level of IFN-γ in each severity group was separated into three different groups; as low, medium, and high serum IFN-γ. Then we realized that these different groups of serum IFN-γ could be used as a cut off for prognosis of infection. The data were reanalyzed after classifying patient groups into low, medium and high IFN-γ levels at <25, 25-85, and >85, respectively. However, there was no correlation between the levels of IFN-γ and any of the severity groups. This suggested that a cut off value of serum IFN-γ, for the immunological severity of HIV outcome during antiretroviral treatment, was not clear and required further investigation.



CD4=cluster of differentiation number 4, sMICA=soluble major histocompatibility complex class I chain related molecule A, ART=antiretroviral therapy

Figure 2 (A) The correlation between IFN-γ (n=40) and CD4 cells in HIV infected patients, with normal response to retroviral therapy, with CD4 decreasing rates less than 35 cell/microliter/year, or viral load lower than 20 copies/milliliter. (B) The correlation between sMICA (n=19) and CD4 cells in HIV infected patients with normal response to retroviral therapy, with CD4 decreasing rates less than 35 cell/microliter/year or viral load lower than 20 copies/milliliter.[2-8]

Discussion

Our study showed that detection of IFN-γ in patients' serum and serum levels of IFN-γ correlated with decreasing CD4 cells, and reduced severe HIV infected outcomes in HIV infected patients undergoing antiretroviral therapy. However, the correlation scores were low. Interestingly, a low correlation may result from the limit of detection of IFN-γ in some patients. Evidence suggested that interference in patient serum may lead to lower detection of IFN-γ or sMICA, via the ELISA technique. Previous reports demonstrated interference from some drug metabolites, soluble receptors, and soluble binding proteins on IFN-γ detection using the ELISA technique.[21-23] In our study, we avoided these interferences by using diluted serum in each testing. Other inflammation or infection conditions may also lead to increasing IFN-γ production and release; however, we also avoided this interference by using patients with no inflammation or opportunistic infection history. With all these criteria, we ensured that the detection of both IFN-γ and sMICA formed immune activation during HIV infection, or antiretroviral drug treatment only.

IFN-γ and sMICA are proteins produced by human immune cells, and may receive some effect from anti-retroviral drugs used to combat HIV infection.[24-26] Previous studies detected increasing IFN-γ production during highly active antiretroviral therapy (HAART) treatment.[27,28] However, one study demonstrated an increase of IFN-γ after HAART therapy, during HIV infection, was detected only inside $CD3^+$ and $CD8^+$ T cells and not released into serum; indicating a low response of T cells.[29] Another major source of IFN-γ is NK cells. Giuliani and colleagues showed a decrease of IFN-γ production in NK cells during long-term HAART treatment.[30] This evidence suggested that long-term antiretroviral therapy may cause a lower response of immune cells involved in HIV responses. This may result in low IFN-γ production, leading to difficulty in detecting disease severity of these patients using IFN-γ levels in serum. However, our results showed promise in using IFN-γ levels to detect severity in HIV infected patients, who had low CD4 decreasing rates. The CD4 cells helped to control T cells, NK cells and IFN-γ production during HAART treatment. Increasing IFN-γ levels in patients with low CD4 decreasing rates correlated with protection of CD4 from destruction by proptosis.[31] Evidence suggested a role of IFN-γ in controlling the immune system, leading to severity of HIV infection. Firstly, Leal and colleague suggested that IFN-γ induced transforming growth factor beta (TGF-β) production, as the result of suppression of NK cells, and transformed them into innate Lymphocyte class 1 inactive cell types.[32] This resulted in lower NK cell activation coupled with lower CD4 cell destruction. Secondly, the effect of IFN-γ on PD-L1 induction has also been demonstrated in some cancer cells. This indicated that higher responses of IFN-γ resulted in inhibition of $CD8^+$ T cell responses, which involved CD4 cell destruction.[33] In brief, after HIV infection the pattern recognition receptor (pRR) expressed on the infected cells; such as, monocyte and dendritic cells were activated by viral components. This activation results in type I IFNs responses; such as, IL-10 and IFN-γ, which effected programmed death ligand 1 (PD-L1) induced expression. Increasing PD-L1 expression led to a protection of virally infected cells from CD8 T cell elimination, due to the interaction of PD-L1 and CD8 T cells, resulting in a blockade of differentiation and the functional process of the cytotoxic T cells.[34]

Another, factor involved in IFN-γ production during infection is the release of sMICA, which inhibits the activation process in both T and NK cells. Production of sMICA is mainly caused by genetic polymorphism. Zigoni and colleague demonstrated specific polymorphism; whereby, MICA-129Val/Val was induced by sMICA production in multiple myeloma patient serum rather than by MICA-129Val/Met and MICA-120Met/Met, respectively.[35] This mutation was detected within the MICA alleles homozygous

A5.1 (MICA*008) variant that was more sensitive to a disintegrin and metalloproteinase, resulting in shedding of MICA from the cell surface.[36] Metalloproteinase has been identified as a major cause of sMICA shedding in many tumors, and infectious diseases. Evidence demonstrated increasing Matrix metalloproteinase 9 (MMP9) expression in patient serum and cerebrospinal fluid in HIV infection with antiretroviral therapy; leading to HIV-1-associated neurocognitive disorders in patients.[37] This information suggested a higher response of MMP9 during treatment with antiretroviral therapy; leading to an increase of sMICA in patients' serum and inhibition of both T and NK cell responses.

Another concordance result was found in Watanabe and college study, which also addressed the negative correlation between IFN-γ and the number of CD4 count in patients with ART treatment.[19] These results suggested the possible use of IFN-γ as a monitoring tool for the immunological suppression in patients with HIV infection during antiretroviral treatment, so as to avoid drug suppress of immune activity. However, this strategy has some limitations. IFN-γ has been suggested for use only in patients with a normal CD4 decreasing rate of lower than 35 cells/year. This major group covered more than 90.0% of patients in antiretroviral treatment in our study, and demonstrated that severity detection using IFN-γ can be applied together with CD4 cell count for patient monitoring. However, detection of IFN-γ in the serum of patients using the in house manual ELISA technique had a lower cost than CD4 count, and testing; therefore it can be performed more frequently. Nevertheless, a cut off value of IFN-γ level is still required for use as an indicator to assess prognosis and treatment success. Therefore, a further study is planned to investigate a larger patient group.

Several studies have demonstrated the use of IFN-γ as a biological marker; for example; as a clearance maker for human papillomavirus, diagnosis tools for congenital toxoplasmosis, and the detection of global loss of T cell function in HIV infected patients with advanced CD4 cell-depleted, for an immune function and opportunistic infection monitoring approaches.[38,39]

However, there are several limitations in this study that must be addressed. Firstly, there was only a small number of patients presenting with IFN-γ or sMICA in serum for using in the correlation analyses. Secondly, a factor contributing to IFN-γ production, during other inflammation and infections, has not been completely ruled out by using only WBC count and patient history; hence, other indications for low grade or sterile inflammations need to be addressed. Thirdly, other patient behaviors; such as, consumption of alcohol and tobacco use might affect patient serum cytokine.[40] Another limitations are that there is no certainty in the cut off value of IFN-γ classification of the higher and lower IFN-γ production groups. Also, a below detectable level of viral RNA in the patient meant that we could not analyze the effect of these biomarkers in a virological effect manner.

Conclusion

The information of this research indicates a negative, moderate correlation between IFN-γ and the severity in patients with human immunodeficiency virus (HIV) infection. This correlation suggests the probability for using IFN-γ as a novel marker for immunological severity detection, for the effect of drugs on the immune cells in the low CD4 decreasing rate group during antiretroviral therapy.

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Conflict of interest

The author declares that there is no conflict of interest.

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