Effect of Structured Physical Activity on Inflammation and Immune Activation Profile of Antiretroviral Therapy-Experienced Children Living With HIV

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Aim: To compare the markers of inflammation and immune activation in virally suppressed HIV-infected children on antiretroviral therapy, who practiced regular structured exercise comprising running and yoga to those who did not over a 2-year period. **Methods:** This retrospective cohort study included 72 children aged 8 to 16 years divided into 2 groups, exercisers ($n = 36$) and the nonexercisers ($n = 36$) based on their intentional physical activity. The analyses were carried out at baseline and after 2 years (Y2) for the soluble biomarkers of inflammation and immune activation (tumor necrosis factor alpha, interleukin-6, interleukin-10, interferon gamma, sCD14, and sCD163). In addition, cell-associated biomarker (CD38), lipopolysaccharides, and the gene expression of interleukin-2 and brain-derived neurotrophic factor were also measured at Y2. **Results:** Reduction in levels of sCD14 (effect size [ES], -0.6 ; 95% confidence interval [CI], -1.08 to -0.14), tumor necrosis factor alpha (ES, -0.7 ; 95% CI, -1.18 to -0.23), interferon gamma (ES, -0.7 ; 95% CI, -1.17 to -0.22), and interleukin-10 (ES, -0.6 ; 95% CI, -1.08 to -0.14) was observed among exercisers as compared with nonexercisers at Y2. In addition, CD38+ expressing CD4+ T cells were found to be lower among exercisers ($P = .01$) at Y2. However, the differences in levels of interleukin-6, sCD163, lipopolysaccharides, interleukin-2, and brain-derived neurotrophic factor were not significantly different among the 2 groups. **Conclusion:** The study result suggests that regular structured physical activity improves the inflammatory profile of antiretroviral therapy-treated HIV-infected children.

Keywords: exercise, running and yoga, soluble biomarkers, tumor necrosis factor alpha, sCD14

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Improved access to diagnosis and widespread use of antiretroviral therapy (ART) has decreased the incidence of opportunistic infection-related morbidity and mortality in individuals living with HIV. However, despite optimal virological suppression with ART non-AIDS defining illnesses (non-AIDS events [NAEs]) encompassing multiple organs are on the rise that increased the incidence of morbidity and mortality in the HIV-infected population (6). The most commonly reported NAEs comprises of non-AIDS defining malignancies, liver, bone, cardiovascular and renal disease, diabetes, and neuropsychiatric disorders (28). The pathogenesis of these illnesses is attributable to the accelerated aging and immune

senescence due to chronic inflammation and immune activation (17) resulting from the direct effect of HIV, microbial translocation, coinfections, and other comorbidities (19).

To reduce the systemic immune activation and NAEs, several therapeutic interventions employing a variety of agents are currently being explored in HIV-infected adults (19). However, as the inflammatory response is a finely tuned process, targeted therapeutic interventions are challenging due to the involvement of various inherent sensors and feedback mechanisms of the immune system (44). In addition, perinatally HIV-infected children differ from their adult counterparts in their immunological, emotional, and psychological manifestations. Therefore, strategies for their long-term management would require a multidisciplinary approach with a focus on the physical, immunological, and emotional/psychological health (4,47).

Regular physical activity is known to improve the physical fitness and mental health status of healthy adolescents (8). Also, physical activity complementing ART interventions is found to be beneficial for adults living with HIV for controlling the chronic inflammation, immune activation, and cardiometabolic health (15). Likewise, yoga therapy has shown to improve the overall well-being and quality of life of HIV-infected adults by relaxing the mind and body and reducing stress (31). Yoga is also known to improve the levels of cortisol, cytokines and brain-derived neurotrophic factor (BDNF), the key molecule for the neurophysiological functioning (10). However, the benefit of regular physical activity and yoga therapy in reducing the inflammation and immune activation is relatively unexplored in ART-treated HIV-infected children (18,29,40).

The authors therefore undertook this study to investigate the effect of a long-term regular structured exercise program comprising running and yoga on the levels of the biomarkers of inflammation, immune activation, microbial translocation, interleukin-2 (IL-2), and BDNF levels in children living with HIV who are on ART.

Materials and Methods

Study Design and Study Population

Participants. This retrospective cohort study assessed the effect of regular structured exercise on the immunological parameters of ART-treated HIV-infected children at 2 different time points: at the study entry (Y0) and after 2 years (Y2). The study participants were selected from a pediatric cohort study that assessed the clinical immunological and virological outcomes in HIV-infected children aged <18 years attending the pediatric infectious disease clinic at St John's Medical College, Bangalore, India, from 2012 to 2018. Children were eligible to be included if (1) aged between 8 and 16 years, (2) they are on ART for a minimum of 6 months at study entry, (3) virologically suppressed (HIV-1 RNA load < 150 copies/mL) throughout the 2-year study period tested at 6-month intervals, (4) no unresolved opportunistic infections reported during the 2-year study period, and (5) completed all the study visits at 6-month intervals for 2 years.

The study was approved by the institutional ethics review board at St John's Medical College (Institutional Ethics Review Board study reference number: 32/2012 to 2018). Written informed consent was obtained from the caregivers, and verbal assent was documented from all children aged 8 years and older prior to participation in the study.

Assessment of Physical Activity. The study participants were assessed for their intentional physical activity behavior using a

physical activity questionnaire suited for Indian children (43) at the study entry retrospectively (Y0) and at the end of year 2 (Y2), prospectively. This questionnaire was further modified to include questions on the habitual physical activity pattern (sedentary and nonsedentary) and included specific questions on yoga and sports and the amount of time spent on each activity per day (Supplementary Material [available online]). From the amount of time spent per week for yoga and sports, the proportion of the year in which the activities were regularly practiced was assessed. Those children who followed a regular exercise schedule for 20 to 45 minutes per day, for a minimum of 4 times per week from Y0 to Y2 were categorized as "exercisers." Those children who were not following any regular exercise or were not engaged in exercise at all, from Y0 to Y2 were included as "nonexercisers." Among the nonexercisers, those children who started practicing a structured exercise program during the study period were excluded. Blood samples obtained from these children were stored in the form of plasma and peripheral blood mononuclear cells (PBMCs) at Y0 and Y2 at -80°C and liquid nitrogen, respectively, until analysis.

Overview of the Exercises Practiced

All the exercisers were from a community care center for HIV-infected children. They practiced a regular structured physical exercise schedule comprising of running and yoga from study entry. Running was practiced in the morning or evening depending on the weather condition for a minimum of 4 times per week under adult supervision. Each running session lasted for 20 to 45 minutes covering a distance of 3 to 10 km for each child depending on the age. In addition, they practiced yoga asanas under supervision during the morning hours for a minimum of 4 times per week for 15 to 30 minutes. Each yoga session consisted of the sun salute (Surya namaskara); the practice of various aligned postures or asanas (Svastikasana, Vajrasana, Trikonasana, Parsvakonasana, Simhasana, Paschimottanasana, Purvottanasana, Pawanmuktasana, Bhujangasana, and Uttanapadasana); controlled breathing (pranayama); and deep relaxation techniques. All the practices (running and yoga) were done under supervision, and compliance was recorded. All the study participants on an average completed 4 sessions per week during the study period. Nonexercisers were children cared for at home, who were not regularly practicing any structured exercise program, yoga asana or any exercise. Both groups reported no intentional physical activity prior to study entry (Y0).

Assessment of Immune Status

Soluble Biomarkers of Inflammation. Pro and anti-inflammatory cytokines including tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6), interferon gamma (IFN γ), and interleukin-10 (IL-10) were measured in the stored frozen plasma at Y0 and Y2 using the enzyme-linked immunosorbent assay according to the manufacturer's protocol (R&D Systems, Minneapolis, MN).

Soluble Biomarkers of Monocyte Activation. Soluble biomarkers of monocyte activation including soluble CD163 (sCD163) and soluble CD14 (sCD14) were measured using enzyme-linked immunosorbent assay in the stored frozen plasma at Y0 and Y2 according to the manufacturer's protocol (R&D Systems). Plasma was diluted 1:20 for sCD163 and 1:1000 for sCD14 assay.

Measurement of Lipopolysaccharides. Plasma lipopolysaccharides (LPS) levels were analyzed using the limulus amoebocyte lysate assay (QCL-1000; Lonza Walkersville Inc, Walkersville, MD)

according to the manufacturer's protocol at Y2. Endotoxin concentrations of the samples were calculated from the absorbance of the standards by linear regression analysis.

Immunophenotyping of Lymphocyte Subsets. Level of T cell activation was analyzed in the cryopreserved unstimulated PBMCs from exercisers ($n = 20$) and nonexercisers ($n = 20$) by multiparameter flow cytometry at Y2. The cells were thawed, washed, cell count adjusted to 1×10^6 cells; surface stained with a cocktail containing live/dead stain (L23101; Invitrogen, Carlsbad, CA), CD3 (340440; BD Biosciences, San Jose, CA), CD8 (347314; BD Biosciences), CD38 (555460; BD Biosciences); and analyzed on a BD FACS Aria II (BD Biosciences). CD3+ T cells were gated from the PBMCs using the forward and side scatter followed by analyzing the CD38 expression on the gated CD8+ T cells. Flow cytometry data were analyzed using the FlowJo software (TreeStar Inc, Ashland, OR). Results were reported as the percentage of CD3+CD8+ cells and CD3+CD4+ cells expressing CD38.

Gene Expression Analysis of IL-2 and BDNF in PBMCs. Gene expression was assessed by measuring the level of IL-2 and BDNF mRNA in the polyclonally stimulated PBMCs at Y2 from exercisers ($n = 20$) and nonexercisers ($n = 20$) with a mixture of phorbol 12-myristate 13-acetate and ionomycin at a concentration of $1 \times$ (cell stimulation cocktail; eBioscience, San Diego, CA). In brief, PBMCs were thawed, washed, cell count adjusted to 2×10^6 cells in Roswell Park Memorial Institute medium supplemented with 10% fetal bovine serum and incubated at 37°C in 5% CO_2 for 6 hours with and without the cell stimulation cocktail. At the end of the incubation period, cell cultures were pelleted, total RNA extracted using QIAamp RNA blood mini kit (QIAGEN, Hilden, Germany) and reverse transcribed using RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific Inc, Waltham, MA) as per the manufacturer's instructions. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the reference gene. The real-time polymerase chain reaction (PCR) for the quantification of mRNAs of IL-2, BDNF, and GAPDH was performed in a Rotor-Gene RG-300 (Corbett Research, Sydney, Australia) cyclor

using DyNAmo ColorFlash SYBR Green qPCR kit (Thermo Fisher Scientific) according to the manufacturer's protocol. The primers described previously were used for the real-time PCR for IL-2 (12), BDNF (24), and GAPDH (23). IL-2 and BDNF signals were normalized with amplified GAPDH signals to compensate for inter-PCR variations between runs (36). The gene expression of IL-2 and BDNF was calculated using the comparative cycle threshold (C_t) method to analyze the relative change in gene expression ($\Delta\Delta C_t$ method) using GAPDH as reference control (39).

Statistical Analysis

All the statistical analysis was performed using the software Stata (version 13; StataCorp, College Station, TX). Statistical significance was set at $P < .05$. Quantitative variables were expressed as frequencies (%) and median (with interquartile ranges [IQRs] at 25th and 75th). Change scores between the groups from baseline to year 2 for cytokine and monocyte activation marker levels were compared using the Kruskal-Wallis H test. The linear regression analysis was performed to assess the association between the baseline value and Y2 change in cytokine and monocyte activation marker levels. Effect size with 95% confidence intervals was calculated to determine the magnitude of the difference between the 2 groups from baseline to Y2 for cytokine and monocyte activation marker levels. Effect size was calculated by dividing the mean difference between the groups with the pooled SD and interpreted according to Cohen, as small (0.2) medium (0.5), and large (0.8) (13). Other parameters were compared using the Student t test or Mann-Whitney U test.

Results

Figure 1 (flowchart) depicts the flow diagram of the selection of 36 exercisers and 36 nonexercisers for this retrospective study. All the included children were perinatally HIV infected.

All the study participants were school-going children belonging to families with low socioeconomic backgrounds residing in the

Figure 1 — Flow diagram of study participant selection. ART indicates antiretroviral therapy.

south Indian states of Karnataka and Tamil Nadu. All the children were on ART containing 2 nucleoside reverse transcriptase inhibitors and 1 nonnucleoside reverse transcriptase inhibitor in World Health Organization clinical stage 1 or 2. The baseline characteristics of the study participants are presented in Table 1.

Effect of Exercise on Soluble Biomarkers of Inflammation and Immune Activation

Soluble biomarkers of inflammation (cytokines: TNF- α , IL-6, IFN γ , and IL-10) and immune activation (sCD163 and sCD14) were measured in blood plasma at Y0 and Y2 in 36 exercisers and 36 nonexercisers. Table 2 demonstrates the comparison between exercisers and nonexercisers in the median change score of the biomarker levels from Y0 to Y2. A significant difference in the change score from Y0 to Y2 was observed between the groups only for sCD14.

To understand how the baseline values affect the estimate of the postexercise (Y2) values, we performed linear regression analysis and calculated the effect sizes as it includes the SD and thereby considers the sample size. Table 3 shows the adjusted mean change score for cytokines and monocyte activation markers between the groups when controlled for the baseline values. This analysis showed a medium effect size for sCD14, TNF- α , IFN γ , and IL-10 in favor of a reduction in levels observed among exercisers compared with the nonexercisers. Small effect size in favor of higher IL-6 level was found among exercisers compared with nonexercisers ($P \geq .05$), whereas the effect was negligible for sCD163.

LPS Concentration

Plasma LPS concentration was measured in blood plasma only at Y2 in 36 exercisers and 36 nonexercisers, as the plasma samples

were not available for each assay at both time points (Y0 and Y2) due to the retrospective nature of the study. No significant difference in the plasma LPS concentration ($\pm$ SD) was observed among exercisers and nonexercisers at Y2 using the limulus amoebocyte lysate assay (0.35 [0.12] EU/mL; 0.36 [0.22] EU/mL; $P = .75$)

CD3+ CD8+ CD38 Expression and Gene Expression Profile

Peripheral blood mononuclear cells were used for the analysis of CD38 expression by flow cytometry and gene expression of IL-2 and BDNF by qPCR. As this was a retrospective study, the availability of PBMCs was limited, and the analysis was performed only at Y2 in the PBMCs available for 20 exercisers and 20 nonexercisers. There was no statistically significant difference observed between exercisers and nonexercisers for the percentage of CD3+CD8+ T cells expressing CD38 at Y2 (median 0.31% [IQR 0.09–0.71] vs 0.46% [IQR 0.28–0.78], $P = .17$). However, the percentage of CD3+CD4+ T cells expressing CD38 was significantly lower among exercisers compared with nonexercisers (median 0.11% [IQR 0.01–0.23] vs 0.24% [IQR 0.16–0.39], $P = .01$). IL-2, BDNF, and GAPDH mRNA were detected in all the samples (exercisers, $n = 20$; nonexercisers, $n = 20$) by real-time PCR checked in duplicate. No significant differences were observed in the relative expression of IL-2 and BDNF mRNA in stimulated or unstimulated PBMCs between the exerciser and nonexerciser group ($P \geq .05$) at Y2.

Discussion

In this retrospective cohort study, we have explored the role of a structured exercise program comprising running and yoga practiced

Table 1 Comparison of Baseline Difference Between Exerciser and Nonexerciser Group

Parameter	Exercisers (n = 36)	Nonexercisers (n = 36)	P
Gender (men), n (%)	27 (75)	16 (44.4)	.08
Age, y	10.5 (9, 12)	12.5 (10, 14)	.06
ART duration, y	3 (1, 5)	3 (2, 5)	.45
CD4 + T cell, %	34.5 (30.2, 40)	31 (28, 37.7)	.13
Body mass index, kg/m ²	14.7 (13.7, 16.1)	15.3 (14.1, 17.1)	.14
HIV-1 RNA load, copies/mL	48 (48, 48)	48 (48, 48)	.64

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range. Note: Values are given as median with IQR.

Table 2 Difference Between the Groups in Change Score for Cytokines and Monocyte Activation Marker Levels From Y0 to Y2 (Unadjusted)

Variables	Exercisers (n = 36)			Nonexercisers (n = 36)			P*
	Y0	Y2	Change score Δ	Y0	Y2	Change score Δ	
IL-10, pg/mL	1.45 (1.02, 1.98)	1.19 (0.78, 1.47)	−0.26	1.67 (1.05, 2.83)	1.85 (1.37, 2.45)	0.18	.09
TNF- α , pg/mL	2.6 (2.1, 3.1)	2.1 (1.9, 2.4)	−0.5	3.14 (3.19, 3.14)	2.98 (1.99, 4.28)	−0.16	.07
IFN γ , pg/mL	0.16 (0.05, 0.69)	0.05 (0.05, 0.35)	−0.11	3.45 (2.56, 5.52)	2.98 (1.99, 4.28)	−0.47	.19
IL-6, pg/mL	1.55 (0.71, 3.34)	1.67 (0.94, 3.91)	0.12	1.12 (0.61, 4.27)	1.36 (0.75, 2.57)	0.24	.4
sCD14, ng/mL	2357 (2104, 2639)	1875 (1655, 2214)	−482	1978 (1575, 2341)	2122 (1724, 2472)	144	.001
sCD163, ng/mL	690 (557, 958)	556 (389, 768)	−134	555 (422, 759)	471 (372, 649)	−84	.1

Abbreviations: IFN γ , interferon gamma; IL-6, interleukin-6; IL-10, interleukin-10; IQR, interquartile range; sCD14, soluble CD14; sCD163, soluble CD163; TNF- α , tumor necrosis factor alpha; Y0, baseline; Y2, year 2. Note: Values are given as median (IQR).

*Kruskal–Wallis H test. $P < .05$ (bold) is considered significant.

Table 3 Comparing the Parameter Scores at the End of 2 Years Between Exercisers and Nonexercisers Adjusted for the Baseline Differences

Parameters	Adjusted mean (SD) ^a year 2		<i>P</i>	Effect size, Cohen <i>d</i>	95% CI
	Exercisers	Nonexercisers			
IL-10, pg/mL	1.3 (1.8)	2.4 (1.8)	.01	−0.6 (medium)	−1.08 to −0.14
TNF-α, pg/mL	2.4 (1.7)	3.6 (1.7)	.004	−0.7 (medium)	−1.18 to −0.23
IFNγ, pg/mL	1.2 (2.0)	2.6 (2.0)	.02	−0.7 (medium)	−1.17 to −0.22
IL-6, pg/mL	2.5 (1.8)	1.8 (1.8)	.1	0.4 (small)	−0.08 to 0.85
sCD14, ng/mL	1888 (433)	2153 (433)	.01	−0.6 (medium)	−1.08 to −0.14
sCD163, ng/mL	559 (225)	564 (225)	.9	−0.02 (trivial)	−0.48 to 0.44

Abbreviations: CI, confidence interval; IFNγ, interferon gamma; IL-6, interleukin-6; IL-10, interleukin-10; sCD14, soluble CD14; sCD163, soluble CD163; TNF-α, tumor necrosis factor alpha. Note: Values that are statistically significant (<.05) are in boldface type. Effect sizes = (adjusted year 2 mean of exercisers − adjusted year 2 mean of nonexercisers)/pooled SD.

^aPostexercise (year 2) mean values adjusted for baseline mean values.

regularly in reducing the HIV-associated inflammation and immune activation in children and adolescents on ART. The study results indicate that regular structured exercise significantly reduces the soluble (sCD14, TNF-α, IFN-γ, and IL-10) and cell-associated (percentage of CD38+CD4+ T cells) inflammatory biomarkers which may have a direct impact on the risk for cardiovascular diseases as well as on the emotional and psychological well-being of HIV-infected children on ART. These findings observed among children are similar to the findings from studies conducted among ART-treated HIV-infected adults (7,35).

We did not find any significant difference among the exercisers and nonexercisers in the levels of plasma soluble CD163, a biomarker for HIV activity in chronic infection. sCD163 is exclusively shed from the activated CD14+CD16+ inflammatory monocytes and tissue macrophages during chronic HIV infection (9). Mediators of inflammation such as LPS and macrophage-mediated diseases increase the plasma levels of sCD163 (9,30). Plasma sCD163 levels also have shown correlation with the percentage of activated CD8+ CD38+ T lymphocytes (9). Bonato et al (7) in their study demonstrated that exercise interventions effectively reduce CD38 expression on CD8+ T cells in ART-treated HIV-infected adults. Our study findings could not demonstrate a similar effect in children, although a significant reduction was observed for the percentage of CD4+ and CD8+ T cells expressing CD38 among the exercisers compared with the nonexercisers. In addition, plasma LPS levels also did not differ among the exercisers and nonexercisers in this study.

In contrast to the sCD163 levels, we observed a significant reduction in sCD14 levels among exercisers compared with the nonexercisers. Studies have shown that successful control of viral replication with ART normalizes the sCD163 levels in both children and adults, although the sCD14 levels remain elevated despite prolonged ART which has prognostic significance (1,11). Thus although HIV viremia is associated with the elevated sCD163 levels, no such association was observed for elevated sCD14 levels (11). Studies have linked the pathways associated with microbial translocation and LPS binding to TLRs expressed on monocytes and macrophages to the elevated sCD14 levels despite prolonged ART (48). However, we found similar sCD163 and LPS levels in both groups and reduced sCD14 levels among exercisers compared with the nonexercisers. Our results may indicate the exercise-mediated reduction in sCD14 levels produced from sources other than monocytes and macrophages such as hepatocytes of the liver. It is known that although sCD163 is produced exclusively by monocytes and

macrophages, the major source of sCD14 in circulation is the hepatocytes and its expression is differentially regulated in liver cells and monocytes (42). Bas et al (5) in their study showed the relation between sCD14 levels and various inflammatory conditions that are not of bacterial origin indicating the possible role of sCD14 besides LPS signaling and described it as an acute-phase protein produced by the liver. Ogawa et al (33) have shown correlation of plasma sCD14 levels with mCD14 expression on Kupffer cells of liver in nonalcoholic fatty liver disease reflecting liver inflammation. Hepatic inflammation during HIV infection is the major predisposing factor for hyperlipidemia, lipodystrophy, metabolic syndrome, and nonalcoholic fatty liver disease; all of which are on the rise in ART-treated HIV-infected population including children (22,32,38). Therefore, our result of the reduction in sCD14 levels among exercisers compared with the nonexercisers suggests the exercise-mediated attenuation of the hepatic inflammatory situation, which can have a direct impact on the metabolic profile and quality of life of HIV-infected children (21).

Studies have shown that, among the various factors associated with inflammation, insulin resistance, and nonalcoholic fatty liver disease the major contributor is the adipose tissue accumulation in the liver (2,46) as the recruitment of activated macrophages to the hypertrophied adipose tissue is associated with higher systemic levels of the proinflammatory cytokines TNF-α, IL-6, and IFN-γ (16). Lihn et al (25) in their study reported an inverse relationship between adiponectin and adipocytokines TNF-α and IL-6 in the pathogenesis of HIV-associated lipodystrophy syndrome, indicating the significance of adipose tissue and its cytokines in HIV infection. Exercise training is known to reduce the visceral fat accumulation in HIV-infected individuals (45) with a concomitant reduction in levels of inflammatory markers including TNF-α and IL-6 (26). We observed a significant reduction in the levels of TNF-α among the exercisers compared with the nonexercisers in this study. However, the IL-6 levels did not differ significantly which is similar to the findings from other studies that showed no significant impact of exercise training on IL-6 levels (20). It has been observed that although ART itself reduces the levels of IL-6, IFN-γ, and IL-10 to the levels seen in uninfected controls, a similar effect is not observed in the case of TNF-α and the levels of which remain elevated even after prolonged ART (3,34,37). Therefore, the reductions in TNF-α and IFN-γ levels observed among the exercisers compared with the nonexercisers may be suggestive of the reduction in adipose tissue inflammation mediated through the practice of routine structured exercise.

Pedro et al (35) have shown that exercise training reduces IL-10 levels in ART-treated HIV-infected adults. Despite the fact that ART itself reduces the IL-10 levels, albeit slowly, due to its potential role in HIV pathogenesis, downregulation of IL-10 activity using immunomodulation therapy (41) and other complementary therapies such as exercise training programs are considered beneficial for chronically infected individuals with HIV for augmenting the partial immune recovery following ART (27,35). Thus, the significant reduction in IL-10 levels observed among the exercisers in this study suggests the immunomodulatory effect of routine exercise training for ART-treated HIV-infected children. IL-2 and BDNF mRNA levels did not differ significantly among exercisers and nonexercisers suggesting no relevant effect of exercise in the gene expression of IL-2 and BDNF in PBMCs.

Overall, this study finding suggests the beneficial effect of a structured exercise program consisting of running and yoga practiced regularly in reducing the inflammatory biomarkers including sCD14, TNF- α , IFN- γ , and IL-10 for ART-treated HIV-infected children. Our results, however, need to be interpreted with caution as the statistical analysis employed effect sizes calculated on nonnormal distributed data.

The advantages of this study include analysis of the long-term benefits of exercise in improving the inflammatory and immune parameters of ART-treated HIV-infected children, which is a unique population and unexplored in the area of exercise physiology. On the other hand, this study has various limitations. First is the usage of blood plasma for the analysis of cytokines that are locally produced molecules with small half life. In addition, the blood samples were obtained at the resting stage (not immediately after exercise) may have contributed to the fact that many of the cytokines were at the lower limit of detection of the assay. In addition, the dietary habits of the children could not be documented on a regular basis due to the retrospective nature of the study. However, all the children in this study belonged to the south Indian states of Karnataka and Tamil Nadu belonging to the lower socioeconomic background whose major diet consists of rice and vegetables. Finally, another limitation of the study is the lack of validation of these results using in vivo/animal models. Given the innate variability of the human immune response, the results may need to be interpreted with caution (14).

Conclusion

Structured exercise comprising running and yoga practiced over a 2-year period is found to be beneficial for ART-treated children infected with HIV, mediated through the reduction in levels of sCD14, TNF- α , IFN γ , and IL-10. The improvement in these inflammatory markers could potentially reduce the risk for CVDs, insulin resistance, and NAEs in HIV-infected children and adolescents on ART. Future studies to explore the long-term impact of regular structured physical activity in the emotional and neuropsychological function of children and adolescents deserve investigation.

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