The Effects of Yeast Selenium on CD4 T Cell Count of Non-Institutionalized HIV type 1 Positive Orphan Children at Orongo Widows and Orphans in Kisumu Kenya

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Abstract

Background: *Multi drug resistance HIV has emerged rendering the current conventional treatment of HIV ineffective. There is a need for new treatment regime which is cheap, effective and not prone to resistance development by HIV.*

Methods: *In randomized clinical study of 68 HIV positive children 3 – 15 years to asses the efficacy of yeast selenium in HIV/AIDS patients, 50µ yeast selenium was administered to 34 children while in matched control of 34 were put on placebo. Blood samples of the both groups which were taken every 3 months intervals up to 6 months, were analyzed by ELISA for CD4T cells, the data was analyzed by SPSS version 16.*

Results: *No significant difference in age { χ^2 (1, 62) =0.03, p =0.853}, cause of morbidity between test and controls {χ^2 (1, 65) = 5.87, p= 0.015} and on condition of foster parents {χ^2 (1,63) = 5.57, p= 0.0172} was observered. Children on selenium showed progressive improvement of WAZ and significant difference at six months{F (5,12) = =5.758, P=0.006}, and weight gain of up to2.5kilograms in six months, and significant CD4 T cell count increase t = -2.943,p< 0.05 compared to matched controls t = -1.258 p> 0.05. CD4 T cell count increased among all age groups on test 3-5years (+ 267.1),5-8 years(+200.3) 9-15 years (+71.2) cells/mm^3 and in matched controls a decrease 3-5 years(-71),5-8 years (-125) and 9-13years(-10.1)cells/mm^3 . No significant difference in CD4 T cell count between girls {F (2, 32) = 1.531 p= 0.232} and between boys {F (2, 49) = 1.040, p= 0.361} on test and between boys and girls {F (5, 81) = 1.379, p= 0.241} on control was observed.*

Conclusion: *From this study it can be concluded that administration of yeast Selenium led to slowing the progress of HIV 1 in children from WHO clinical stage I by improving CD4 T cell count and hence the immunity.*

1.0 Introduction

There is evidence that strongly suggests that prevalence of HIV type 1 is linked to selenium deficiency in diet. HIV positive patients suffer extreme deficiency of selenium and that of cystein, tryptophan and glutamine which are components of enzyme Glutathione peroxidase. As HIV replicates it depletes selenium and the three amino acids (Mariorino *et al,* 1998).Evidence has emerged showing that HIV positive patients gradually become deficient of selenium which in turn compromises immune response to the infection (Baum *et al,* 1979).

It has been observed that serum selenium levels is a better predictor of mortality than CD4 cell count(Foster,2000).The observations tend to suggest that HIV replicates much faster in selenium deficient communities, where immunity to HIV is already compromised. Selenium has been observed to be key in prolonging health for persons living with HIV (Foster, 2000). Deficiency of selenium has been observed to lead to increased mortality among HIV positive patients (Kupka *et al,* 2004, Constans *et al,* 1995).Selenium's role as antioxidant and in immunity could be the underlying mechanism. The role of selenium in HIV positive patient's progression is better assessed by a randomized control study. Several studies done in USA showed that selenium supplementation reduced rate of hospital admissions (Bethony *et al,* 2006) and suppressed viral loads (Hurwitz *et al,* 2007).But effect of selenium in populations in Sub-Sahara Africa is not known. This study is therefore being conducted as randomized controlled trial of selenium on HIV + infected children at Orongo Widows and Orphans in Kisumu Kenya. Several factors affect bioavailability of selenium from the diet, hence to asses the actual clinical effect a random controlled clinical study of HIV positive patients using a known quantity can indicate its' actual immunological effect which is reflected either as improved nutritional status WAZ or in improved CD4T Cell count and % or both. This study was designed to investigate the immunological benefit of administering selenium to HIV positive patients in form of CD4 T cell count and CD4 T Cell % and WHO clinical disease staging.

2.0 Objectives of the Study

The general objective of the study was to determine the effects of increased selenium intake amongst HIV-1 positive patients. Specifically the study was to determine the effects of selenium administration to the HIV positive Patients on CD4 T cell count and weight for age Z Score .

3.0 Research Methods

3.1 Study Area and Study Population

The study site is located 4 kilometers from Kisumu Town centre along Kisumu Nairobi road in Nyamasaria area. Town has an average population of 332,000, which are divided into four administrative divisions, Kombewa, Kadibo, Maseno and Winam. The study took place in Nyamasaria which is in Winam Division in Kisumu Municipality with a total population of 184,243 and HIV prevalence of 14.9 %(NASCOP, 2005). The study population is mainly petty traders and fish mongers. The HIV/AIDS prevalence is twice the National Prevalence but factors leading to this persistent high differential prevalence are not fully understood. The study subjects were the orphan children, enrolled at Orongo Widows and Orphans in, Kisumu, which were tested and those found to be HIV positive were selected to join the study.

3.2 Study Design

This was a prospective, randomized clinical trial designed to determine the effects of yeast selenium on disease progression of HIV-1 positive non institutionalized orphan children at Orongo Widows and Orphans. The children are registered at Orongo Dispensary located in vicinity of the centre hence their clinical conditions including WHO disease staging were ascertained.

3.3 Sampling Procedure

The children meeting criteria indicated below were assigned numbers, and using and using computer generated random numbers the required number was chosen after consultation with authorities at the centre. The sample size was determined by formula suggested by Epi Info version 6 for cohort studies in which the required minimum sample size at 95% Confident interval and precision of 80%, and prevalence of HIV amongst the study subjects 15%(KAIS 2007), Relative Risk of 4.4 % the number in test group is given as 21 while for the controls at 21 the minimum sample size being 42.

3.4 Inclusion Criteria

The first criterion of selection was that the study subject had to be HIV positive. Since CD4 cells are central to the function of immune system their level was used to monitor the AIDS progression (pathogenesis).The CD4T cell count was done at Nyumbani Childrens Home. Those on stage 3 and below were selected, this minimized chances of losing study subjects during the course of research due to early progressors. This also reduced complications during the course of study due to opportunistic infections. The second criteria of selection was that the children are 3 years and above but below 15 years.

The fourth criteria were that the guardians have given consent for the children to participate in the study. Finally they were also required to be attending clinic with clear records as a symptomatic.

3.5 Exclusion Criteria

The first exclusion criteria were the children were HIV negative. The second was that the guardians had not given consent, while the fourth criteria were that the they are in WHO stage 4 and above. While the final exclusion criterion was that the children are less than 3 years and more than 15 years.

3.6 Randomization

Each of the children was assigned a number. Using computer generated random numbers the first 33 children were chosen and these formed the test group while the remaining group of 33 formed the control group which was put on placebo.

3.7 Ethical Consideration

Approval to conduct the study was given by Kenyatta National Hospital Ethics Research and Standard committee. All patients who met the criteria were given equal chance to be enrolled in the study. Informed consent was given by the guardians/parents and the information collected was kept confidential.

3.8 Blood Sample Collection and Laboratory Analysis

At baseline, trained research assistants collected data of childrens' socioeconomic and health status. The participants on test then received 50µg yeast selenium .The dose is about a half of tolerable upper limit for children which are 100µg (yeast selenium) per day. Every week a visit was made to the home to check on progress of the children and at 3 months intervals, the research assistants collected data on weight and Blood samples to measure immune status (CD4-T cell count).

The blood samples (2ml) to measure immunologic parameters which are CD4-T Cell counts were collected by the researcher, in vacuutainers with anticoagulant. Complete CD4 T- cell count was evaluated among children at baseline and subsequently every 3 months, up to six months.CD4T-Cell count was then quantified by way of ELIS A method.

The samples were put on a roller and then CD4 T cell count done in Guava® Auto CD4/CD4% System as follows:- in a vial, 10µL of Guava®CD4/CD4% auto cocktail was added, this was followed by 10µL blood from a patient, the mixture was incubated for about 30 minutes in darkness. To the mixture was added 380µL of Guava lyse solution, this mixture was further incubated for 15 minutes in darkness. The fluorescent labeled sample was aspirated through a proportioned micro-capillary flow cell. A green laser excited the cells and each cell emitted a signal which was individually detected by photomultipliers and photoiodide. The system allows absolute cell count without reference beads (Millipore, 2009) .

3.9 The Study Outcome

The primary study outcome which was the change in CD4T cell count, and Weight was determined by comparing change after subsequent reading by χ^2 , student t analysis at 95% confidence interval and by Z-Score. The secondary out come was qualitative assessment of general health of the patients by use of Karnofsky score.

4.0 Statistical Analysis

Compliance with the study regime was calculated as number of tablets absent from the bottles supplied divided by tablets supplied-Score was calculated to asses any improvement on the children anthropometry. ANOVA was used to compare the changes in CD4 T- cell count. The regression analysis was done of CD4 T Cell count as dependent variable on age, gender and Z-score of patients as;

$Y= \Sigma\ \alpha+\beta_1 X_1 + \beta_2 X_2 +\varepsilon$

Where y is dependent variable CD4 T cell Count

X_1 is independent variable like age, gender, nutritional status (WAZ)

ε The error term taking care of all other damy variables which affects the outcome such as genetic variation, psychosocial factors etc.

Further tests were done by χ^2 analysis and student t test.

P-value presented is two sided statistical significance of 0.05 or below. Analysis was carried out using SPSS version 16.00(SPSS, Inc. Chicago, IL, USA) and Epi info version 6.While means are values + or – SD unless stated. Comparison of change group of CD4 cell count within group (treatment) and between groups' treatment and counts was done at 95% confident interval using student t test.

5.0 Results

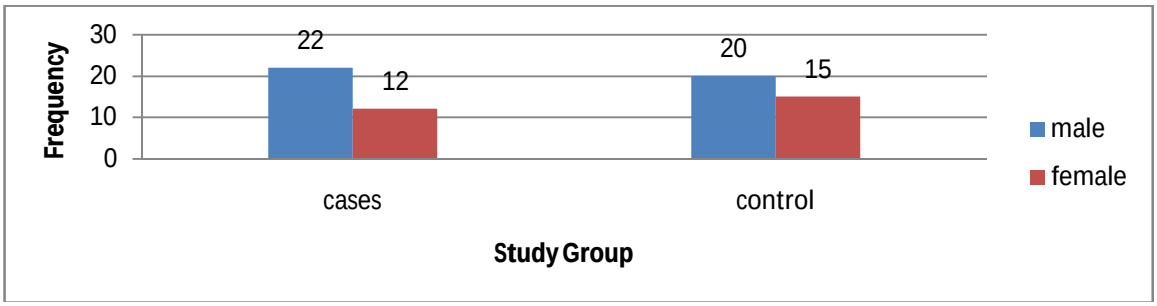
5.1 Demographic Characteristics of the Study Population

5.1.1 Age and Gender Distribution of the Study Subjects

There was no significant difference in age distribution amongst the study population was observed {χ^2 (1,62) p=0.185}.The mean age was 7.7±3.4 years for the test group, while for the matched controls had the mean of 8.8±3.2 years.

Gender distribution shows that 64.7% of all children on test were boys while 35.3% were girls. In the matched controls 54.05% were boys and 44.95% were girls further analysis show there was significant differences in the distribution of gender between the test and control groups{ χ^2 (1,62) =0.03 p= 0.853}.This is further illustrated in the figure 1 below.

Figure 1.Gender Distribution of the Study Subjects



5.1.2 Economic Status of Foster Parents

In the study it was shown that among the test group 30% of study subjects reported being worse off in their foster homes than in their original households, while remaining were either same (30%) or better off (40%).How ever the control group 87.5% of study subjects reported being better off with their foster parents than in the original homes table 6 below. But the difference in condition of both the two groups was not significant { χ^2 (1 N=63) =5.65, p=0.0172}. 5

5.1.3 Cause of Morbidity and Mortality of the Study Population

In the two groups most of the childrens' parents are reported to have died from various conditions but with chronic diseases and HIV being reported as being the main cause of mortality with mortality of their fathers not being significantly different(p = 1.00). However analysis shows that there was significant difference in cause of mortality of mothers between children on test and those on controls {χ^2 (1, N=63), p= 26.00,p =0.00).

The children enrolled in the study reported having suffered from various acute and chronic conditions mostly HIV/AIDS related in the previous twelve months as shown in table 1 below. There was slight significant difference in various health conditions suffered between the children on test and those in controls {χ^2 (1, N=64) = 5.78, P= 0.015}.They also reported having been treated in various health facilities.

Table 1: Medical and Socio Economic Conditions of the Children and their Parents

Medical Conditions	Test Group	Control Group	χ^2	P-Value	RR
Morbidity of children					
Loss of weight/Diarrhea /Chronic fever	15	27	5.87	0.00	
Skin Rush/Others	16	8.0	-	- -	
Cause of Fathers' Death					
Chronic Conditions/HIV	32	31	-	1.00 -	-
Cause of Mothers' Death					
Chronic Conditions/HIV	30	11	26.00	0.00	4.40
Others	1.0	20	-	-	- -
Economic Status of foster homes					
Better off/same	22	30	5.67	0.0172	
Worse off	9.0	2.0	- -	-- -	-

5.2 Effect of Selenium Administration on CD4T Cell Count

5.2.1 Changes in CD4 T Cell Counts among Different Age Groups

There was increase in mean CD4 T cell counts compared to baseline among the test subjects in all age categories at six months; 3-5years from 1031.7±716.1cells/µL to 1298.8±812.9cells/µL(+267.11),6-8years from 1300.0±468.5cells/µL to 1500.3± 440.4 cells/µL(+200),9-15years 928.2± 450.8cells/µL to 989.4±485cells/µL (+61) (table 6).

In the matched controls there was a decrease in CD4T Cell counts in all age categories. 3-5 years from 1670±706.3cells/µL to 1599±532 cells/µL (-71), 6-8 years from 1340±446cells/µL to 1215±119.2cells/µL(-125),9-15years1271±364.1cells/µL to1260.9±515cells/µL (-10.2) this is further shown in table 6 below.

Table 2: Change in Mean CD4 T cell Count (cells/µL) by Age

	3-5yrs		6-8yrs		9-15yrs----------	
	Mean	95% CI	Mean	95% CI	Mean	95%CI-------
(Test Group)						
Baseline	1031.7	(305, 2298)	1300.0	(877, 1984)	928.2	(356, 1879)
3 Months	1244.0	(350, 2237)	1372.7	(830, 1853)	868.8	(360, 1664)
6 Months	1298.8	(450, 2299)	1500.3	(878, 1993)	989.4	(370, 1889)
(Control group)						
Baseline	1670.0	(1276, 2919)	1340.4	(922, 2367)	1271.1	(449, 1489)
3Months	1554.0	(833, 2248)	1215.1	(1004, 1430)	1237.0	(815, 2215)
6Months	1599.0	(987, 2356)	1215.0	(1002, 1359)	1260.9	(851, 2371)

5.2.2 Changes in CD4 T cell Count by Gender (Sex of Respondents)

Analysis of variances show that there was no significant differences in CD4 T cell between girls on test at six months{F (df 2,32) =1.531, p = 0.232}.Similarly no significant differences in CD4 T count was observed between boys{ F (df 2,49) =1.040, p=0.361} and between boys and girls {F(5,81) =1.379 p=0.241) among the test group. In the matched controls there was no significant difference in CD4T cell count between different gender {F (df 5,86) = 1.168 p= 0.332} at six months.

5.2.3 The Change in CD4 T cell Counts (Paired t Tests)

The mean CD4 T cell count increased from 1029.8(±543.1) cells/µL to 1197.4(±613.5)cells/µL(+168) , at six months, this was significantly different between the second and third sampling {t(1, N=60) = -6.1, p= 0.000}, and between first and third sampling {t (1, N=30) =-2.9, p= 0.006) among the children on test (table 14).On the matched controls there was a decline of mean CD4 T cell count from 1836.5(±2249.2)cells/µL to 1313.4 (±393.6)cells/µL(-523),but no significant difference between the second and third sampling{ t =(1, N=30) 1.8, p= 0.078} and between first and third sampling {t(1,29) = -1.3 p=0.218} was noted(table 6).

Table 3: Paired t test of the Mean CD4 T Cell Count

Selenium Group

Paired Test	Mean CD4 T Cell(all ages)	t value	P value
CD4T cell 1 CD4T cell 2	1029.8(±543.1) 1097.4(±592.4)	-1.209	P= 0.236
CD4T cell 1 CD4T cell 3	1029.8(±543.1) 1197.4(±613.5)	-2.943	P= 0.006
CD4T cell 2 CD4T cell 3	1097.4(±543.1) 1197.4(±613.5)	-6.075	P= 0.000

Control Group

Paired Test	Mean CD4 T cell Count(all ages)	t value	P value
CD4T cell 1 CD4T cell 2	1836.5(±2249.2) 1284.8(±387.7)	-1.309	0.201
CD4T cell 1 CD4T cell 3	1836.5(±2249.2) 1313.4(±393.6)	-1.258	0.218
CD4T cell 2 CD4T cell 3	1284.5(±387.7) 1313.4(±393.6)	1.828	0.078

5.2.4 The Effects of Treatment on Mean CD4 T Cell Count Change

Change in the mean of CD4T Cell count between baseline and six months sampling was found to be significant among the test group at 95% confidence interval {F (2, 27) = 4.65 p= 0.0183}, while in the matched controls no significant difference was observed at six months {F (2, 23) =0.40 p=0.6742}.There was significant difference in CD4 cell count between test group and the matched controls {F_t /F_c (df 30,25)11.625 p< 0.05}.

5.2.5 Correlation between CD4 T cell count, Z-Score for Weight and Gender

In the model above multiple regression analysis of the correlation between absolute CD4T cell count ,sex and weight for age (WAZ score) established that change Z-score for weight was positively correlated to change in CD4 cell count for the children on test {t(2,27) = 2.94,p=0.007} with R = 0.2523 and adjusted R^2 being 0.2016, while in the matched controls there was no significant correlation between weight for age, and CD4 cell count observed {t (2,23) =0.08 p=0.934} with R being 0.0337 while adjusted R^2 being -0.0503 (table 12 below). In the regression model when gender was factored, the analysis shows that in the test group, it was not significantly correlated to the change in CD4 T cell count {t (2 ,27) = -0.69 p= 0.495}.In the matched controls similarly the gender was not significant {t (2, 26) = - 0. 90, p= 0.380}.

Table 4: Correlations between CD4 T Cell Count and WAZ Scores

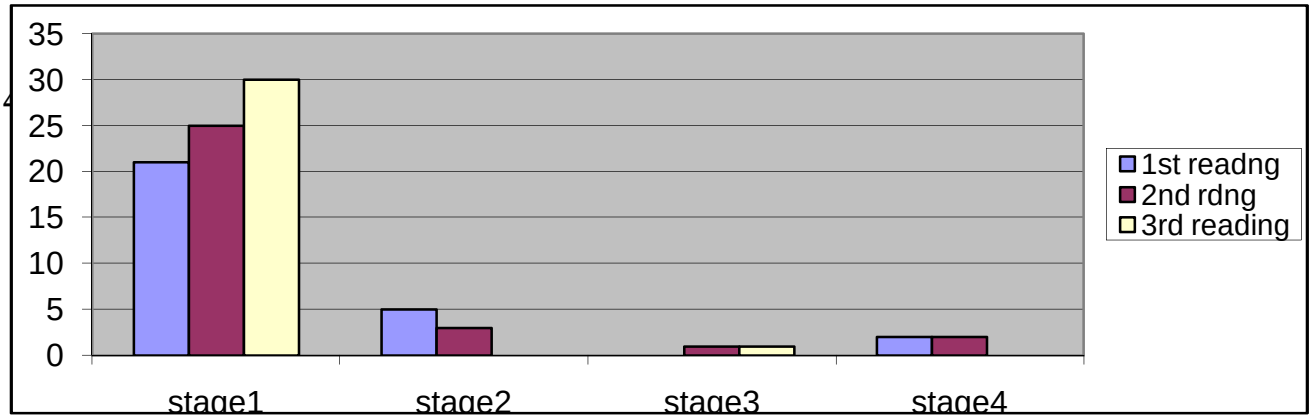
	B	Std. Error	R^2.	t	Sig.
Test					
WAZ	252.2307	85.89016	0.2523	2.94	0.007
Control					
WAZ	3.366466	40.42608	0.0337	0.08	0.934

In the regression model, $CD4count=\beta_0+\beta_1 sex+\beta_2 WAZ+\varepsilon$, the gradients of correlation of the two variables gender (sex),weight for age (WAZ) and CD4 T Cell count varied. In the test group WAZ score interaction with change in CD4 T cell count, the gradient coefficient was positive (β_2 = 252.23),while interaction of gender (sex) with change in CD4 T cell count, the coefficient was negative(β_1 = -138.23).In the matched control group the WAZ interaction with change in the CD4T cell count the coefficient was only slightly positive (β_2 = 3.366) while the gender(sex) interaction with change in CD4 T cell count the coefficient was negative (β_1 = -135.50).

5.3 Effect of Selenium Administration on Quality Of Life (QoL) of Study Subjects 5.3.1 WHO Immunological Disease Staging (test group)

Most (73%) of the study test subjects were in WHO clinical stage one at baseline as shown in the table 13 below. At second reading 80.64% of the children were found to be in stage I while at third reading majority 96.78% of the study subjects had progressed to stage I figure 8.

Figure 2: Clinical Staging



5.3.2 Karnofsky Score Estimates of the study subjects

2(6.5%) children scored 80%. In the matched controls 30(96.8%) of the children scored
90 while the remaining 1 (3.2%) scored 80 %.At six months the score it was estimated that 31(100%) of the children on test were in Karnofsky score of 100 while of the matched controls 31(100%) had declined to the score of 80%.

6.1 Discussion

In this randomized clinical trial, yeast selenium capsules 50µgms were given to HIV positive children (ages from 3yrs to 13yrs) at Orongo Widows and orphans in Kisumu Kenya. The demographic characteristics and socio-economic factors which were established at baseline, of both the groups were found not to be different. Most of the children in both groups were found to be paternal orphans. While the cause of the fathers' deaths were found to be either HIV/AIDS(74%) or chronic condition(94%) likely to be HIV/ AIDS related in all cases, which suggests that the most of the children were infected vertically either in utero or at birth. It also points to high HIV prevalence in this community as it is known that only 30-40% of children born to HIV positive parents get vertically infected (NASCOP, 2002).

Significant differences in CD4 T cell count calculated at six months was noted among the group on selenium, as compared to the controls where there was no significant difference noted. This was an indicative of an improving immunity ($p< 0.05$) amongst the children on test compared to the matched controls ($p> 0.05$).The typical CD4T count for a healthy child (1-12 years old) is between 500-2500 cells/μL^{-1} (Foster 2006). In HIV-positive patients not receiving ARVs, the CD4 count decreases on average between 50 to 100 cells μL^{-1}.In this study an increase of up to 267 cells/µL was observed (in 3 to 5years age group) on test at six months while a decrease of 125 cells/µL was observed (in children 6-8 years old) amongst the controls.

Earlier studies show that the level of CD4 T cell count is directly affected by the level and activity of seleno-protein GSH-px (Beltz *et al,* 1991; Hiscott *et al,* 2001), while level of GSH-px correlates well with the level of selenium in the blood (Debski *et al,* 1989, Meltzer *et al,* 1993).The measure of CD4 T cells therefore is a surrogate measure of the level of selenium in the body which is as a result of selenium intake. The increase of CD4T cell count in this study therefore suggests that selenium intake improves the immunity of the HIV positive children in the study site and hence a factor in determining the progression of HIV positive children to AIDS.

These findings tend to support several studies which have suggested beneficial effects of selenium to HIV positive patients (Hurwitz *et al* ,2007; Kupka *et al,* 2005,; Campa *et al,* 1999) have shown association between low selenium levels in Childs' blood and reduction in hospitalization .

Selenium in the body influences both cell mediated and humoral immunity (Burbano, *et al* ,2002),and expression of receptors of interleukin-2(IL-2)which influences cytotoxic T-Lymphocyte activity (Kremidjian-Schumacher *et al*, 2002) and increases interferon yield and T-Cell count. Absence of selenium leads to CD4 T Cell apoptosis and increased HIV replication through activation of Nuclear Factor kappa B by unchecked increase of $H_2 O_2$ activity. Other studies have shown relationship between patient survival and selenium levels in blood (Baum *et al*, 1997,Campa *et al,* 1999).

A study in Miami in 2002 did examine the effects of selenium administration to adult men and women over a 9 month period, showed that increased selenium in the body is associated with decreased HIV load in the body and improvement of CD4 cell count (Hurwitz *et al,* 2002).How ever in these studies it is not clear weather confounding factors which include concurrent intake of antiretroviral drugs could have influenced the outcome.
In this study improvement of children to WHO clinical Stage I was observed which an indicative of improved immunity. This has been observed in earlier adult researches cited above. Karnofsky score initially developed by Karnofsky and Burchenal (1949) was used to measure for general improvement of health and physical well being of the patients. In this study it was observed that most of children on test had improved to scale 100 of the Karnofsky score estimates, while in the controls most study subjects were at 80% by six months.

This suggested improvement of quality of life amongst the children on test which can be attributed to selenium intake. This study contributes to the literature on understanding of the effects of selenium administration to HIV positive children. The yeast selenium provided is commonly available in chemists as nutritional supplements. From this study it is observed that selenium supplementation leads to increase in CD4 cell count ($p< 0.05$ between baseline and six month sampling, and between second and six month) and delayed progression of the AIDS as seen by improvement in WHO clinical staging. The weight gain of up to 2.5 kilograms and improvement of Z-Score which is significant at 5%.

7.0 Conclusion and Recommendations

7.1.1 Summary of Conclusions

Using data generated from this study, rigorous analysis has shown positive impacts of selenium administration to HIV positive individuals in resource limited setting makes and important contribution to the evidence in the role of selenium administration in management of HIV/AIDS positive patients. In this study it can be concluded that Administering yeast selenium to HIV positive children (3-15years) led to the increase of mean CD4T cell count amongst all age groups from (1029.8±543.1)to (1179.4±613.10) of the test subjects in six months period suggesting selenium administration improved immunity.

7.1.1 Recommendations

Based on the findings above it is be recommended that;

1 Yeast Selenium should be given as nutritional supplement for a symptomatic HIV/AIDS infected patients to improve on CD4 T cell count hence immunity and delay the progress to clinical Stage IV.
2 Further research is necessary to determine the following;

(i) Possible environmental impact of long term use of selenium for fortification of foods.
(ii) Cost effectiveness of using direct yeast selenium supplementation as compared to promoting selenium rich foods.
(iii) Earliest Clinical stage to introduce yeast selenium as supplement to HIV positive patients to delay progress to stage IV.

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