HIV-infected adolescents on anti-retroviral therapy: a retrospective descriptive cohort study of breast abnormalities documented during routine care

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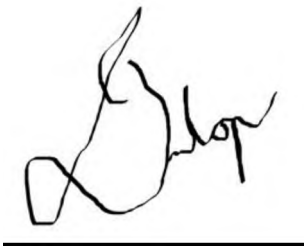
Breast abnormalities in adolescents on ART

Declaration:

I Jackie Dunlop declare that this Research Report is my own, unaided work. It is submitted for the Degree of Child Health at the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(Jackie Dunlop)

20th day of **June 2017** in **Johannesburg**

Presentations arising from this research project

- South African HIV Clinician's Society Conference, April 2016, Sandton Convention Centre, Poster
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Abstract

Background:

HIV antiretroviral therapy (ART) is associated with breast abnormalities in adults, especially efavirenz (EFV). Little is known about the prevalence of these adverse effects among adolescents receiving ART.

Methods:

A retrospective record review describing breast conditions in adolescents receiving ART at three facilities in Johannesburg, South Africa was conducted. Patients aged 10-19 years who presented from 1 January to 31 December 2014 were included. Analyses were conducted to determine whether EFV use was associated with an increase in breast conditions.

Results:

A total of 631 patient records were reviewed, 37 (6%) had an abnormal breast event documented of whom 24/37 (65%) were male. Patients with abnormal breast conditions developed them 1.5 years later than patients with normal breast development ($p<0.0005$). Forty-one abnormal breast events were observed in thirty-seven patients with twenty being described as gynaecomastia or lipomastia (49%). 44% had concurrent generalised lipodystrophy (n=19). Of those with an abnormal breast event, 71% of patients had CD_4 counts >500 cells/µl and were virologically suppressed (n=29). Those on EFV had a significantly higher prevalence of breast abnormalities compared to other regimens ($p=0.016$) and all had been exposed to EFV before. No other ART drug was associated with breast abnormalities in this cohort. Sixteen patients had substitution of EFV and three breast events resolved once substituted from EFV.

Conclusion:

Six percent of patients had an abnormal breast condition in this study. Use of EFV and increasing age were associated with breast abnormalities in this population. Further research is needed to better understand this phenomenon.

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Acronyms

ABC: abacavir

ART: antiretroviral therapy

ARV: antiretroviral

ATV: atazanavir

AZT: zidovudine

BMI: body mass index

D4T: stavudine

DDI: didanosine

DRV/r: darunavir/ritonavir

EFV: efavirenz

FTC: emtricitabine

HIV: Human Immunodeficiency Virus

LM: lamivudine monotherapy

LPV/r: lopinavir/ritonavir

NRTI: nucleoside reverse transcriptase inhibitor

NNRTI: non-nucleoside reverse transcriptase inhibitor

NVP: nevirapine

PI: protease Inhibitor

RPV: rilpivirine

SD: standard deviation

TDF: tenofovir disoproxil fumarate

VL: viral load

3TC: lamivudine

1. Background

Increasing numbers of HIV-infected children are entering into adolescence. Thus, ensuring appropriate care, including the recognition and management of co-morbidities of HIV and the side effects of antiretroviral therapy (ART) is a vital aspect of HIV management. Increasing numbers of adolescents on ART, both male and female, are reporting abnormalities of their developing or, already developed, breasts.[1]

Adolescence is a time of profound physical development and is defined by the World Health Organisation as individuals aged 10 to 19 years.[2] During this period, puberty leads to changes in previously childlike bodies, and breast development in girls, as well as in boys, is part of typical pubertal development.[3–5] This development in girls is well described by the Tanner staging system.[3] Here the development of a normal breast is described from the initial tiny breast bud to become a mature adult breast.[3]

One described problem of breast development in teenaged girls is breast hyperplasia.[4] Breast hyperplasia or hypertrophy in adult women receiving efavirenz (EFV) has been described however the same condition is not well explored in adolescent girls.[6] Juvenile breast hyperplasia is defined as the uncontrolled overgrowth of breast tissue that occurs in adolescents whose breasts develop normally during puberty, however fail to stop growing at the appropriate time.[4]

Tanner staging does not account for normal pubertal-related, physiological breast enlargement or gynaecomastia in boys.[3,5] Gynaecomastia is defined as glandular proliferation causing enlargement of the male breast. Physiological gynaecomastia is described in three periods of male life: during the neonatal period, adolescence and in the latter years.[5] In one large cross sectional study, physiological gynaecomastia

was shown to present in 4% of boys aged 10 to 19 years old.[5] Physiological gynaecomastia may present in boys as young as 10 years old, however, the peak onset in adolescence is usually between 13 and 14 years old.[5] The postulated cause for this is not known though hormonal fluctuations during adolescence may contribute.[5] Causes of pathological gynaecomastia include medications, adrenal and testicular neoplasms, congenital syndromes such as Klinefelters and Peutz-Jeghers, hyperthyroidism and thyrotoxicosis, liver failure and cirrhosis, primary hypogonadism, congenital adrenal hyperplasia, androgen insensitivity, obesity and malnutrition.[5]

Pathological gynaecomastia in adolescence due to excess oestrogen caused by certain medications has been described.[5,7] In particular, these are attributed to the use of medications for cardiovascular disorders (amiodarone and calcium channel blockers), asthma (theophylline), central nervous system disorders (phenytoin and tricyclic antidepressants), tuberculosis (isoniazid) and other infections (ketoconazole and metronidazole).[7]

Antiretrovirals, particularly EFV, have been shown to cause breast abnormalities in adults.[6,8] The breast abnormalities attributed to antiretroviral therapy (ART) are divided into two classes: the first being lipomastia associated with lipodystrophy and the second, breast enlargement in those patients who have no evidence of lipodystrophy.[8] Little is known about the prevalence of these adverse effects in adolescents receiving ART.

Lipodystrophy can be caused by different ARVs particularly nucleotide reverse transcriptase inhibitors (NRTI) such as stavudine (D4T) and zidovudine (AZT).[9] The role of efavirenz (EFV) and lopinavir (LPV) remains disputed however in some cases

these drugs have been linked to the development of lipodystrophy.[9] Sonography may be used to differentiate between lipomastia related to lipodystrophy and true gynaecomastia in males on ART.[8] Breast ultrasonography was shown to be a sensitive method of distinguishing an increase in ductal tissue and periductal stroma associated with gynaecomastia from the predominantly fatty accumulation seen in lipomastia.[8]

It has been shown that EFV, a non-nucleotide reverse transcriptase inhibitor (NNRTI), is capable of activating oestrogen receptors and causing growth of breast tissue.[10] The effects of this oestrogen receptor activation in a child or an adolescent undergoing puberty are largely unknown.

Research done on the UK and Ireland's national Collaborative HIV Paediatric Study (CHIPS) cohort, studied the frequency, cause and management of severe gynaecomastia.[1] In that cohort, 3% experienced gynaecomastia (n=56/1873) of which 10 patients were reported to be severe.[1] The median age of onset for these patients was 13.5 years and they had been on their current ART regimen for a median of 27.5 months.[1] Half of the affected patients were receiving an NNRTI and the other half a Protease Inhibitor (PI), however all ten had previous exposure to EFV, didanosine (DDI) and D4T.[1]

Furthermore, a case study from South Africa described a further case of a prepubertal girl who developed pseudogynaecomastia four months after initiation onto EFV-based ART with full resolution of the breast enlargement following cessation of EFV.[11]

In another case of a 15 year old HIV infected boy, severe bilateral breast development occurred two years after changing to a regimen containing D4T, DDI

and EFV.[12] He was virologically suppressed at the time.[12] Regression of the breasts did not occur after drug substitution and a bilateral mastectomy was performed.[12]

This is in contrast to the findings in adults, which usually support a shorter temporal relationship of a few months to developing gynaecomastia or breast enlargement once starting a new EFV-containing regimen.[6,13] In a case series originating in Bordeaux, France, two woman and four men experienced breast pain and/or enlargement within one to six months of switching to an EFV-based regimen.[6] In all six patients hormone profiles were normal and they remained on EFV while in only one case did spontaneous regression occur.[6] In the same study, 28.7% of the 258 patients receiving ART were on EFV and, of these, 8,1% had experienced breast enlargement.[6] This includes the six patients described above.

Another group in the United Kingdom suggest that gynaecomastia in HIV infected adults can be as a result of immune reconstitution inflammatory syndrome (IRIS).[8] IRIS is defined as a clinical syndrome consistent with an inflammatory process with evidence of an immune response which occurs usually within three months of ART initiation.[14] IRIS generally occurs in a subset of HIV-infected individuals when ART is initiated at low CD_4 counts and HIV viral replication is halted.[14,15] This leads to a patient's CD_4 count increasing, causing an inflammatory reaction to previously undetected pathogens in their body.[15] This inflammatory reaction was hypothesised to cause gynaecomastia in a small group of 15 patients where no association with EFV treatment was seen as patients were treated with different classes of ARVs.[8] Once full immune reconstitution had taken place, resolution of the gynaecomastia occurred in 80% of the patients.[8]

It has been hypothesised that many cases of gynaecomastia are caused by hypogonadism.[16] A study performed at two HIV referral centres in Barcelona, Spain, suggested that most cases of gynaecomastia in HIV infected male patients occurred as a result of hypogonadism related to HIV infection itself rather than as an adverse effect of ARVs.[16] The hypothesis was strengthened by the fact that both affected and control patients had a similar length of exposure to D4T, AZT and EFV, whereas the affected group had significantly lower free testosterone indices when compared to the control group (p=0.006).[16] However the findings of other studies suggest no difference in the hormonal profiles of the participants.[8,13] A limitation of this study was that hypogonadism was not uncommon in the control group which was not explained by the methodology of the study.[16]

Interventions for breast abnormalities in HIV infected adults differ according to the hypothesised cause. In the Spanish group who hypothesised, that gynaecomastia was secondary to hypogonadism, managing the hypogonadism was key.[16] Patients with gynaecomastia had a mean free testosterone index of 42.6% compared to the controls with 58.1% (p=0.006).[16] They described that most cases of gynaecomastia resolved spontaneously and, for those who did not resolve, intramuscular or transdermal testosterone was administered.[16] The gynaecomastia response to the testosterone replacement are not described in the published study.[16]

If the hypothesis that EFV causes breast abnormalities by activating oestrogen receptors is accepted, then the use of anti-oestrogen drugs such as Tamoxifen and the substitution of EFV are recommended.[5,10] In the child and adolescent cohort in the United Kingdom, optimal management of severe breast abnormalities ranged varied from an immediate ART substitution to differing periods of observation which led to a change in ART for most patients.[1]

No treatment was deemed necessary where IRIS was the accepted hypothesis.[8] In the UK adult cohort, once the immune reconstitution had occurred and the cytokines began to fall, spontaneous resolution of the gynaecomastia was noted.[8] Where fat accumulation as part of lipodystrophy was seen concurrently with breast enlargement, drug substitution of the PI was recommended.[9] In most cases, this was not possible, and, even where possible, it was not highly successful.[9] In these cases surgical intervention, with its accompanying risks, was seen as the only remaining option.[9] There was, however, a risk of recurrence of fat accumulation in the breasts with up to a half of patients experiencing a recurrence within one to two years of surgical intervention.[9]

Therefore, the aim of this study was to establish the extent of breast abnormalities in adolescents receiving ART in South Africa and to determine whether there were associated ART drugs, co-morbidities, common investigations and prescribed interventions in this setting.

2. Methods

2.1. Record review and data extraction

Study Type:

A retrospective review of routinely collected medical records was performed at three public health facilities managing adolescent HIV patients in Johannesburg, South Africa.

Inclusion criteria:

In order for patients' records to be eligible for review, they had to be aged 10 to 19 years, on ART and had to have presented to their health facility during the study period, 1 January to 31 December 2014.

Exclusion Criteria:

Records that did not meet these criteria, or were not available on site (for example lost or archived files) were not included in the review.

Study Sample

All available files meeting the inclusion criteria were reviewed.

Data capture and storage:

For eligible files, information regarding patient's demographic profiles and a brief ART history were captured. The current ART regimen during the study period was recorded where possible for all eligible records.

Patient records were then screened for mention of the words "breast", "gynaecomastia" or "lipomastia". For those with a recorded breast-related event, HIV information, both around the time of the breast event, as well as a comprehensive history of ART regimens was captured. A detailed clinical history around the time of the event as well as details of the actual breast condition were also captured.

The description of the breast as well as whether the breast changes were considered to be normal development or an abnormal condition were captured in freehand to ensure no important information was lost.

Data collection forms were developed specifically to extract all relevant information for this study from the existing clinical records. All recorded patient information was de-identified and given a study number to maintain confidentiality. A password protected linking file was kept. Study data was then managed using REDCap electronic data capture tools hosted at University of Witwatersrand.[17]

2.2. Outcome of Interest

The outcome of interest was whether an adolescent patient on ART had an abnormal breast event. For patient files with any breast event mentioned, the breast events were categorised as abnormal or normal. Abnormal events were defined prior to the study as being inclusive of descriptions of gynaecomastia, lipomastia, abnormal breast enlargement and breast lump. The "normal" breast cases were grouped together with those in which there was no mention of breast who were assumed to be developing normally as well.

Information from the records regarding whether an abnormal breast condition was investigated or intervened upon were analysed and the nature of the interventions for each abnormality were categorised. For those who received an ART drug substitution, the use of different drugs as well whether a particular drug substitution led to resolution of the breast problem was analysed.

2.3. Data collection: Patient demographic, HIV and breast-related clinical information

All eligible medical files were included in the data analysis. For all eligible records, demographic data were analysed. These included age (in years) at time of last visit

during study period, clinic where patient received treatment including the approved study sites of Alexandra Health Care Centre and University Clinic (Clinic A), Right to Care Paediatric HIV Clinic (Clinic B), Witkoppen Health and Welfare Centre (Clinic C) and sex of the patient. Patient ART history analysed included age (in years) at ART initiation, duration on ART at time of last visit during study period, and current regimen at time of last visit during study period. Table 1 shows the different ART drugs available in the public sector for use in children and adolescents in South Africa, the class to which each drug belongs and the associated adverse events.

Table 1: Individual antiretroviral drugs for paediatric and adolescent treatment in South Africa

Antiretroviral	Class	Formulation	Adverse effects
Abacavir (ABC)	NRTI	Solution 20mg/ml, Tablets 60 mg, 300 mg, ABC/3TC	ABC hypersensitivity reaction
Atazanavir (ATV)	PI	Tablet 150mg, 200mg, 300mg	Indirect hyperbilirubinaemia, ECG abnormalities
Darunavir (DRV)	PI	Tablets 75mg, 150mg, 600mg	Hepatitis, Rashes
Didanosine (DDI)	NRTI	Tablets 25mg, 50mg, 100mg,Capsule 250mg	Lactic Acidosis
Efavirenz (EFV)	NNRTI	Capsules 50mg, 200mg, Tablet 600mg	Persistent CNS toxicity, Hepatotoxicity
Emtricitabine (FTC)	NRTI	Co-formulated with TDF and TDF+EFV	Skin, hypersensitivity reactions
Lamivudine (3TC)	NRTI	Solution 10mg/ml, Tablet 150mg, 300mg	Rare, Pure red cell aplasia
Lopinavir/r (LPV/r)	PI	Solution 80/20mg/ml, Tablets 200/50mg, 100/25mg	Gastrointestinal, Dyslipidaemia, Liver enzyme elevation
Nevirapine (NVP)	NNRTI	Solution 10mg/ml, Tablet 200mg	Hepatotoxicity, Hypersensitivity Reaction, Convulsions
Rilpivirine (RPV)	NNRTI	Tablet 25mg	Rashes, Liver Failure
Ritonavir (r)	PI	Solution 80mg/ml, Tablet 100mg	Gastrointestinal, Hyperlipidaemia
Stavudine (D4T)	NRTI	Solution 1mg/ml, Capsule 15mg, 20mg, 30mg	Lipodystrophy, Lactic Acidosis, Peripheral neuropathy
Tenofovir (TDF)	NRTI	Tablet 300mg, Co-formulated with FTC and FTC+EFV	Acute and chronic renal insufficiency
Zidovudine (AZT)	NRTI	Solution 10mg/ml, Tablets 300mg, Co-formulated with AZT	Lipodystrophy, Anaemia, Neutropaenia

Adapted from the National Consolidated Guidelines of the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children, adolescents and adults and the Guidelines for the Management of HIV in Children, 2010 [18,19]

Drugs not included in the national guidelines include darunavir and rilpivirine[20,21]

For each file, the current ART data was analysed by comparing whole regimens as well as the use of individual ART drugs prescribed. Table 2 shows the common ART regimens used in South Africa since the rollout of the national ART programme in 2004.[22] As can be seen in table 2, all regimens contain two NRTI drugs, with lamivudine or, its analogue emtricitabine, being present in all but two previously used second line regimens and Lamivudine Monotherapy (LM). All regimens also contain either a PI or an NNRTI with no recommended regimens containing both. LM contains neither a NNRTI nor a PI. Lamivudine Monotherapy (LM) is an alternative to a suppressive ART regimen and was often used as a holding regimen in this cohort.

All the drugs in the NRTI class were compared looking for the NRTI drugs that were most often prescribed in this cohort (excluding lamivudine, which is present in almost all prescribed ART regimens for children and adolescents).

The use of drugs in the NNRTI and PI classes in this cohort were compared. NNRTI and PI drugs are grouped together as usually only one drug from either class is present in a first or second line regimen at a time.

Table 2: ART regimens for paediatric and adolescent treatment in South Africa

Regimen	Indicated ages	Indication	Guidelines
D4T + 3TC + LPV/r	6 months – 3 years	First line	2004 – 2010
D4T + 3TC + EFV	> 3 years (and > 10kg)	First line	2004 – 2010
ABC + 3TC + LPV/r	<3 years	First line	2009 – to date
ABC + 3TC + EFV	3 - 14 years	First line	2009 – to date
TDF + FTC + EFV	≥ 15 years	First line	2015 – to date*
AZT + DDI + NVP	6 months - 3 years	Second line	2004 – 2010
AZT + DDI + LPV/r	All children failing and EFV based regimen	Second line	2004 – 2010
AZT + 3TC/ABC + LPV/r	All children failing an EFV based regimen	Second line	2010 – 2013
3TC monotherapy (LM)**	In children failing a first line regimen that are unable to take a second line regimen	Holding regimen	Currently

** Treatment of adolescents was first mentioned in the 2010 Clinical Guidelines for the management of HIV & AIDS in adults and adolescents however the age groups for using different regimens, TDF over ABC, was only defined in the 2015 National Consolidated Guidelines*[18,23]

***Not formally included in the South African ART guidelines however is used widely.*[24]

For those with abnormal breast events, the total number of ART regimens patients had received were analysed. Other data analysed included baseline CD_4 count and HIV-1 viral load (VL), all ART regimens prescribed, CD_4 counts and HIV-1 VL at each regimen change, reasons for changing each regimen and the duration of each regimen. A change in ART regimen was defined as a change in any ARV in an adolescent's regimen. This, however, did not include dosage adjustments or a change in the same drug's formulation.

At the time of the first reporting of a breast abnormality, the total duration on ART as well as the length of time on the current regimen, ARV drugs prescribed as part of

the current regimen, the CD_4 count and HIV-1 VL results (±3 months from the date that the breast event was first recorded) and the reported adherence were analysed. Reported adherence was categorised into none (<10% of doses taken), a few (10 – 30% of doses taken), about half (30-60% of doses taken), most (60-90% of doses taken) and all (>90% of all doses taken). CD_4 results were grouped for ease of analysis into four groups: <200 cells/µl; 200-500 cells/µl; 501-1000 cells/µl; >1000 cells/µl. HIV-1 VL results were similarly grouped into those considered to be suppressed at less than 50 copies/ml, those 50-1000 copies/ml and HIV-1 viral load results over 1000 copies/ml.

With regards to particular ARV drugs, the use of different individual drugs was compared for those with abnormal and normal breasts. Significant relationships between individual ART drugs and breast abnormalities were sought and significance established using the Fisher's exact test of difference of proportions. For all abnormal breast cases, EFV and D4T exposure was tabulated as well as the median time of exposure to both calculated.

Clinical information surrounding the event such as pubertal development including Tanner staging, anthropometric measures particularly body mass index (BMI) (kg/m^2), and details of investigations and interventions performed were analysed. Existing co-morbidities including lipodystrophy (fat accumulation and lipoatrophy), hyperthyroidism, hypogonadism, obesity, liver failure and renal failure were specifically considered however any concurrent medical condition was included in the analysis. Concomitant medication use was also explored and the particular medications prescribed were analysed. The presenting breast condition and description of the breast as well as investigations and interventions for abnormal breast conditions were included in the analysis.

2.4. Data Analysis

All statistical analysis was done using STATA version 14 (Stata Corporation, College Station, Texas, USA).[25] Medians and interquartile ranges were used to report descriptive statistics where appropriate. The prevalence of breast abnormality in this population of HIV-infected adolescents on ART was determined. Cases were categorised according to patient demographics, HIV and ART characteristics including the particular ART drugs and clinical measures around the time of the breast event. Statistically significant differences in the prevalence of breast abnormality by demographic and ART data were established.

Statistically significant associations between the patient characteristics extracted and abnormal breast conditions were tested using a Fisher's exact test for difference in proportions (if count or categorized variable) and the Student's t-test for difference of means (if continuous variable). Fisher's exact test was chosen over the Pearson chi-squared test owing to the small numbers with breast conditions. A probability value of $p<0.05$ was designated for statistical significance.

2.5. Ethical considerations

Ethical clearance to perform this retrospective record review was sought from the University of Witwatersrand Human Research Ethics Committee (M141134). As data was collected retrospectively from medical records and no patient contact or patient follow-up was needed, informed consent for the participants of this study was not requested. There was no risk of patient confidentiality being breached as data collection forms contained no identifying information. There were no direct benefits to patients whose files were included in this study. Indirect benefits may be available as more information about managing breast conditions in adolescents on ART may be part of the outcome of this study.

3. Results

3.1. Patient characteristics

The number of records that were screened equalled 1376 and out of those 631 were eligible for inclusion in the study (45.9%). The basic demographics of the files reviewed are shown in table 3 (n=631). The median age for this study population was 14.0 years (IQR: 11.9 -16.0 years). More than half of the eligible files were from Clinic A (53.9%) and the rest were from Clinic B and C. There were more females in the cohort (1.05:1).

Table 3: All patients' characteristics

Characteristics	Description	Count (%) (n=631)
Age	Median (IQR) years	14.0 (11.9-16.0)
Clinic	Clinic A	340 (53.9%)
	Clinic B	102 (16.2%)
	Clinic C	189 (30.0%)
Sex	Male	304 (48.2%)
	Female	322 (51.0%)
	Not recorded	5 (0.8%)

3.2. Patient HIV history

The regimens of only 554 files were available to be retrieved electronically. Collecting this data had not been part of the initial data collection plan for patients without breast abnormalities and therefore these details had be captured from the clinic electronic patient records once the manual file review was completed. As electronic capturing was not complete, not all regimens were available. All patients

with breast abnormalities, however, did have their ART regimens captured during the initial file review.

Details of ART regimens as well as individual ART drug exposures of all eligible patient files are shown in table 4 to 6. The most common regimen was a combination of ABC, 3TC and EFV (n=363, 65.5%). The next most commonly used regimen was LM (n=64, 11.6%).

3TC was the most commonly used NRTI in this population, with 96.2% of patients receiving 3TC or its analogue FTC as part of their regimen (n=533/554). ABC was the next most frequently used NRTI with 72.0% of patients receiving an ABC-containing regimen at the time of review (n=399/554). TDF is the third most commonly used NRTI (n=58, 10.5%) and the mean age of patients receiving this regimen was 16.8 years (Standard deviation (SD): 2.3 years) which was statistically significantly older than the rest of the studied cohort at the time of file review (n=13.8 years, SD: 2.4; $p<0.0005$). This was followed by AZT (n=45, 8.1%) and D4T (n=11, 2.0%).

The majority of patients were receiving NNRTI-based regimens (n=421, 76.0%) when compared to PI-based regimens (n=60, 10.8%). The commonest NNRTI in use is EFV with 418 patients receiving it as part of combination ART at the time of censure (75.5%). The other NNRTI which was routinely in use was NVP (n=2, 0.4%) with one patient receiving RPV (0.2%).

With regards to PI regimens, 50 patients were receiving LPV/r (9.0%), the most common PI in use, and nine patients were receiving atazanavir/ritonavir (ATV/r) (1.6%) with only one patient on darunavir/ritonavir (DRV/r) (0.2%).

Table 4: ART regimen for at the time of review for included records where regimen was retrieved (n=554)

ART Regimen	Count (%) n=554
ABC+3TC+EFV	363 (65.5)
LM	64 (11.6)
TDF+FTC/3TC+EFV	40 (7.2)
ABC+AZT+LPV/r	17 (3.1)
AZT+3TC+LPV/r	16 (2.9)
ABC+3TC+LPV/r	9 (1.6)
Other / Incomplete*	45 (8.1)

**Regimens that are not described in table 2 or have not been frequently used have been placed under Other/Incomplete.*

Table 5: NRTI antiretroviral at the time of review for included records where regimen was retrieved

ART	Description	Count (%) (n=554)
NRTI*	ABC	375 (67.7)
	AZT	21 (3.8)
	D4T	10 (1.8)
	TDF	51 (9.2)
	ABC & D4T	1 (0.2)
	ABC & AZT	19 (3.4)
	ABC & TDF	2 (0.4)
	ABC & DDI	1 (0.4)
	D4T & DDI	1 (0.2)
	TDF & AZT	4 (0.7)
	ABC,TDF,AZT	1 (0.2)
	No other NRTI (includes LM**)	68 (12.3)

**Lamivudine excluded as part of a suppressive ART regimen*

***Number of patients receiving LM = 64*

Table 6: NNRTI or PI antiretroviral at the time of review for included records where regimen was retrieved (n=554)

ART	Description	Count (%) (n=554)
NNRTI	Total	421 (76.0)
	EFV	418 (75.5)
	NVP	2 (0.4)
	RPV	1 (0.2)
PI	Total	60 (10.8)
	LPV/r	50 (9.0)
	ATV/r	9 (1.6)
	DRV/r	1 (0.2)
Both	NNRTI + PI	4 (0.7)
Neither (includes LM)		69 (12.5)

3.3. Prevalence of breast abnormalities

The number of files that reported any breast event was 52 (8.2%). Some patients had breast mentioned multiple times in the file with the total number of breast events, both normal and abnormal, adding to 60 for 52 patients of which 68.3% (41/60) of breast events were described as abnormal in 37 patients. The prevalence of the breast abnormalities reported in the cohort evaluated was 5.9% (n=37/631).

Breast events that were separated by a period of six months in the patient's record, without mention in between, were considered to be separate events.

3.4. Demographic characteristics for abnormal breast events

Table 7 shows the patient and breast characteristics for those files in which abnormal breast events are recorded. Most abnormal breast events were reported in patients 10-15 years of age (68.3%, n=28). In our study population, only two patients

under 10 years old experienced breast abnormalities whereas 11 patients between 15 and 19 years old experienced breast complaints.

Table 7: Demographic characteristics for breast events (n=41)

Characteristic	Description	Count (%)
Age at breast event (n=41)	median (IQR) years	13.5 (12.1 - 15.6)
	< 10 years	2 (4.9%)
	10 - 15 years	28 (68.3%)
	> 15 years	11 (26.8%)

3.5. HIV and ART characteristics for abnormal breast events

Table 8 looks at the HIV patient characteristics for those who had abnormal breast events reported. All patients with recorded abnormal breast conditions were on ART at the time of the reported event. Only 27.0% of patients with breast abnormalities had been exposed to three or more ART regimens prior to developing their breast condition (n=10/37) while the remaining 73.0% had received two regimens or less (n=27/37). The majority of patients had been taking antiretrovirals for three years or more (73.2%, n=30) and most had only been on their current regimen for a median of two years with the shortest time being 0.3 years or 112 days. An excess of two thirds of patients had CD_4 counts higher than 500 (70.7%, n=29) and were virologically suppressed as defined by a viral load of 50 copies/ml or fewer (70.7%, n=29). Good adherence to ART, defined as patient or patient caregiver self-report that greater than 90% of doses were administered, was noted in 63.4% of the patients with abnormal breast conditions with five patients having defaulted ART at the time of breast abnormality.

Table 8: Patient HIV and ART characteristics for abnormal breast events (n=41)

Characteristic	Description	Count (%)
Years on ART from initiation to breast event	median (IQR) = 4.9 (1.8 - 6.6)	
	< 1	3 (7.3)
	1	8 (19.5)
	2	2 (4.9)
	3	3 (7.3)
	≥4	25 (61.0)
Years on regimen leading to breast event	median (IQR) = 2.0 (1.2 - 4.6)	
	<1	7 (17.1)
	1	12 (29.3)
	2	6 (14.6)
	3	5 (12.2)
	≥4	11 (26.8)
CD_4 count at breast event (cells/µl)*	median (IQR) = 708.0 (439.0 - 957.0)	
	<200	4 (9.8)
	200 – 500	8 (19.5)
	500 – 1000	21 (51.2)
	>1000	8 (19.5)
HIV-1 Viral load at breast event (copies/ml)*	median (IQR)	176.5 (91.5 - 373.5)
	Undetectable (<50)	29 (70.7)
	Viral load 50 – 1000	10 (24.4)
	Viral load >1000	2 (4.9)
Reported adherence to ART	None recorded	10 (24.4)
	Good adherence	26 (63.4)
	Lost to follow up	5 (12.2)

** Defined as test taken +/- three months from the visit date when breast event is recorded*

3.6. Clinical and breast characteristics for abnormal breast events

Table 9 shows that there was a lack of information recorded about the pubertal stage of the adolescents with only four patients (9.8%) with abnormal breast conditions having Tanner staging mentioned in their file. Only one patient was recorded to have passed through menarche.

Three patients were reported to have normal breast development, one male and two females, which was later, at another visit, thought to be abnormal. With regards to the condition reported, enlargement of the breasts was most often recorded with 18 cases of abnormal breasts receiving this description. Thirteen had a diagnosis of gynaecomastia, four a diagnosis of lipomastia and three a diagnosis of both lipomastia and gynaecomastia recorded in their file. Other recorded conditions were breast buds in two male patients and breast lump was noted in a single patient. For one patient there was no description of the breast/s recorded.

In 25 patients with abnormal breast conditions, there was no severity of the breast condition recorded in the file. For the remainder, two had early or small as their abnormal breast severity, eleven mild or moderate and only three were described as large or severe.

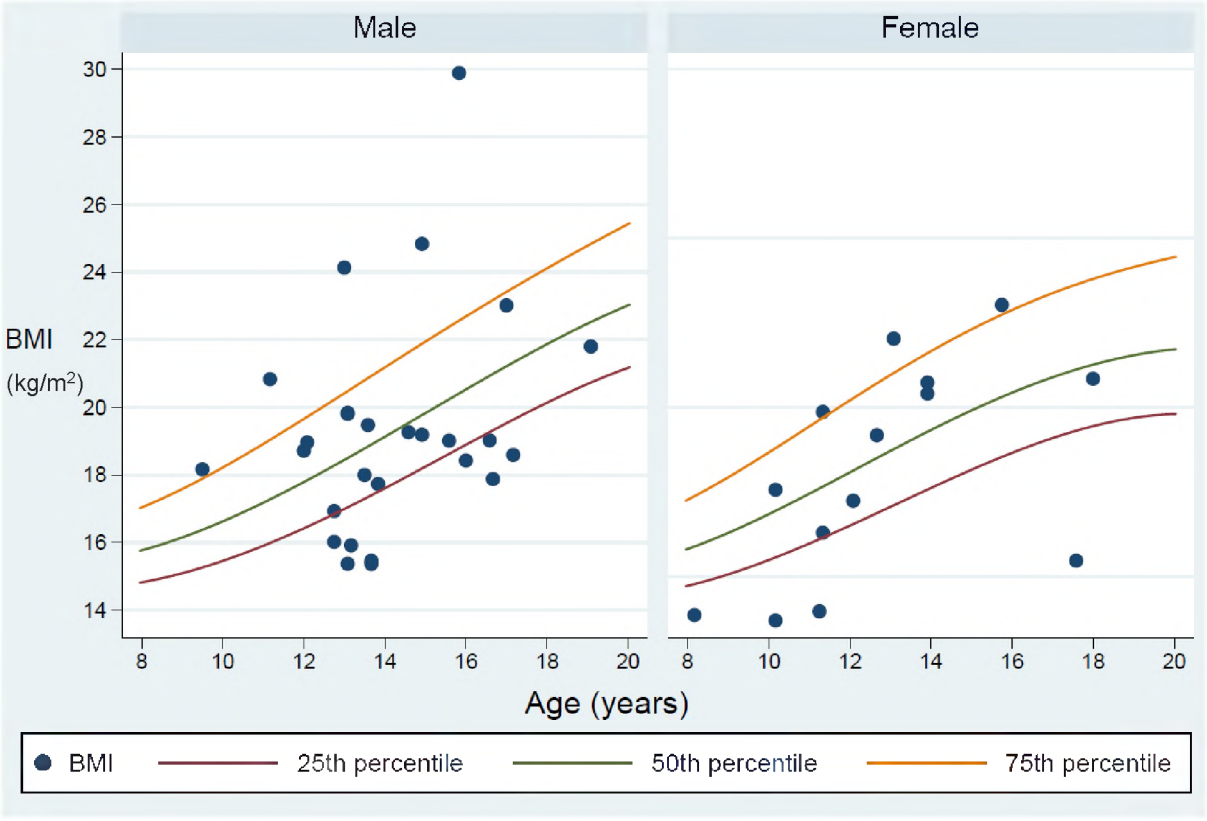
Eight of the patients were described as having bilateral breast changes whereas only five had a unilateral breast condition with 28 having no description of whether a single breast or both breasts were affected.

Table 9: Clinical information for abnormal breast events (n=41)

Characteristic	Description	Count (%) (n=41)
BMI (kg/m^2)	median (IQR)	19.0 (16.9 - 20.4)
	Underweight (<25th percentile)	12 (29.2%)
	Normal (25th-75th percentile)	21 (51.2%)
	Overweight (>75th percentile)	8 (19.5%)
Tanner staging recorded	Yes	4 (9.8%)
	Normal breast development	3 (7.3%)
	Menarche	1 (2.4%)
Description of breast	Enlargement	18 (43.9%)
	Breast buds	2 (4.9%)
	Gynaecomastia	12 (29.2%)
	Lipomastia	4 (9.8%)
	Gynaecomastia/ Lipomastia	3 (7.3%)
	Breast lump	1 (2.4%)
	None recorded	1 (2.4%)
Severity of breast condition*	Early / small	2 (4.9%)
	Moderate / mild	11 (26.8%)
	Severe / very large	3 (7.3%)
	None recorded	25 (61.0%)
Unilateral / Bilateral	Unilateral	5 (12.2%)
	Bilateral	8 (19.5%)
	None recorded	28 (68.3%)

** Only single counts for each event taken, in case of multiple description of size from clinician, the largest size is taken*

Figure 1 shows few patients were reported to be tending towards being overweight (n=8/41, 19.5%) which was defined as a BMI plotting over the 75th centile. Most having a normal BMI (n=21, 48.8%), BMI between the 25th and 75th centiles or being underweight, less than the 25th centile (n=12/41, 31.7%).



Data for figure 1 was sourced from the Centers of Disease Control and Prevention website.[26]

Figure 1: BMI (kg/m2) scatter plot for breast events reported in male and female adolescents on ART (Male: n=27; Female: n=14)

3.7. Association of breast abnormalities to patient characteristics

Table 10 shows the characteristics of those patient files that had documented abnormal breast events compared to files that had normal breast or no mention of breast development or conditions. Patient age at the latest visit in the study period was significantly different, with those with breast abnormalities being older than the group with normal breasts ($p<0.0005$). There was no difference observed in the incidence of breast abnormalities across all three sites ($p=0.622$). There was a statistically significant difference in the prevalence of abnormal breast across sex with males reporting a higher prevalence (7.9%) compared to females (4.0%) ($p=0.043$).

Table 10: Association of breast abnormalities to patient demographics (n=631)

	Characteristics	Patients with abnormal breast (n=37)	Patients with normal breast (n=594)	p-value
Age (years)	During follow-up Mean (SD)	15.5 (2.0)	14.0 (2.6)	**<0.0005**
	At ART initiation Mean (SD)	8.3 (4.2)	8.5 (4.3)	0.771
Clinic	Clinic A	19 (51.4%)	321 (54.1%)	0.622
	Clinic B	8 (21.6%)	94 (15.8%)	
	Clinic C	10 (27.0%)	179 (30.1%)	
Sex	Female	13 (35.1%)	309 (52.0%)	**0.043**
	Male	24 (64.9%)	280 (47.1%)	

3.8. Association of breast abnormalities to ART characteristics

Table 11 and 12 show the differences in ART drugs prescribed in those with abnormal breast events compared to those without breast concerns. In both groups, most patients were receiving ABC, combined with 3TC as their NRTI backbone. Among patients treated with ABC, 7.5% had a reported breast abnormality however this was not significant (n=28/375; p=0.363).

Table 11: NRTI antiretroviral for patients at the time of first abnormal breast event and for patients with no mention of breast or normal breast events (n=554)

ART	Description	Total (n=554)*	Abnormal (n=37) Count (%)	Normal or no mention (n=517) Count (%)	p value*
NRTI	ABC	375	28 (7.5)	347 (92.5)	0.363
	AZT	21	1 (4.8)	20 (95.2)	0.998
	D4T	10	2 (20)	8 (80)	0.139
	TDF	51	2 (3.9)	49 (96.1)	0.563
	ABC & D4T	1	0 (0)	1 (100)	0.998
	ABC & AZT	19	0 (0)	19 (100)	0.630
	ABC & TDF	2	1 (50)	1 (50)	0.129
	ABC & DDI	1	0 (0)	1 (100)	0.998
	D4T & DDI	1	1 (100)	0 (0)	0.067
	TDF & AZT	4	0 (0)	4 (100)	0.998
	ABC,TDF,AZT	1	0 (0)	1 (100)	0.998
	Incomplete / other**	68	2 (2.9)	66 (97.1)	0.296

** Excluded those records for which regimen was not available (n=77)*

***Lamivudine excluded as part of a suppressive ART regimen*

Among the 418 patients on EFV, there were 34 patients with abnormal breast events (8.1%), a statistically significant association (p=0.016). Of the remaining patients, two abnormal breast events occurred on LM with one occurring on an NRTI-only holding regimen. This prevalence of 4.3% was lower than the overall prevalence of abnormal breast events in the cohort but the difference was not statistically significant. For those patients taking LPV/r, no breast abnormalities were recorded and breast abnormalities were more commonly seen with other drugs (p=0.039).

Table 12: NNRTI or PI for patients at the time of first abnormal breast event and for patients with no mention or normal breast events (n=554)

Drug class	Antiretroviral	Total (n=554)	Abnormal (n=37) Count (%)	Normal or no mention (n=517) Count (%)	p value
NNRTI	EFV	418	34 (8.1)	384 (91.9)	**0.016**
	NVP	2	0	2 (100)	0.998
	RPV	1	0	1 (100)	0.998
PI	LPV/r	50	0	50 (100)	**0.039**
	ATV/r	9	0	9 (100)	0.998
	DRV/r	1	0	1 (100)	0.998
Both	NNRTI + PI	4	0 (0)	4 (100)	0.998
Neither*		69	3 (4.3)	66 (95.7)	0.606

**Neither includes LM*

Particular exposure to chosen ART drugs is shown in table 13. All patients with abnormal breast conditions had received EFV as part of a prior or current regimen. The median time of exposure to EFV was 5.5 years. Comparatively, almost 60% of those with abnormal breast conditions had been exposed to D4T with the median time of exposure of 4.9 years.

Table 13: EFV exposure for those patients with abnormal breast (n=37)

EFV exposure	Count (%)	37 (100%)
Accumulative time on EFV based regimen*	median (IQR)	5.5yrs (3.8-8.5)
D4T exposure	Count (%)	22 (59.5%)
Accumulative time on D4T based regimen**	median (IQR)	4.9yrs (1.8-7.2)

** Measured up until first breast abnormality/change from EFV*
*** Measured up until change from D4T*

3.9. Management of adverse event

Table 14 shows the concomitant medication, comorbidities, investigations and interventions for patients with abnormal breast conditions. One or more concomitant medication was received by 61.0% of patients (n=25) and 73.8% had a co-morbidity (n=30), with over half having two or more comorbidities (n=23, 56.1%).

Co-morbid lipodystrophy was diagnosed in 46.3% of those with abnormal breast conditions (n=19), with nine of those patients being classified as having fat accumulation syndrome (22.0%).

Only three of the patients were investigated for their abnormal breast condition. One patient received sonography and two others had blood taken to be analysed. Of these, one was tested for their prolactin levels with no result recorded.

Nearly half of the patients received no intervention for their abnormal breast condition. The most common intervention was the substitution of EFV for another antiretroviral (n=16, 39.0%). In six instances other ART substitutions took place either at the same time as changing from EFV or separately. Diet and exercise and Tamoxifen, an anti-oestrogen drug, were prescribed for one patient each. Three

patients were seen at a specialised breast clinic and there were plans to refer a further two patients in the next year. Almost half of abnormal breast conditions received no intervention (n=20, 48.8%).

Table 14: Concomitant medication, comorbidities, investigations and interventions for abnormal breast events

Characteristic	Description	Count (%) (n=41)
Concomitant medications	None	16 (39.0)
	At least one drug	7 (17.1)
	2 - 4 drugs	15 (36.6)
	5 drugs	3 (7.3)
Co-morbidities	No comorbidities	11 (26.8)
	Only 1 comorbidity	7 (17.1)
	2 - 4 comorbidities	18 (43.9)
	5 comorbidities	5 (12.2)
Lipodystrophy syndrome	Total with lipodystrophy	19 (46.3)
	Lipoatrophy	6 (14.6)
	Fat accumulation (FA)	9 (22.0)
	No description	4 (9.8)
Investigations	Investigation requested	3 (7.3)
	Sonography	1 (2.4)
	Blood tests	2 (4.8)
Interventions	No prescribed intervention	20 (48.8)
	Intervention prescribed	21 (51.2)
	EFV substitution	13 (31.7)
	Other ART change	3 (7.3)
	EFV and other substitution	3 (7.3)
	Diet and exercise	1 (2.4)
	Tamoxifen prescribed*	1 (2.4)

**Tamoxifen prescribed but not received (taken from the clinical notes)*

Of the 19 patients who received an ART drug substitution, table 15 shows details of the drug substitution and whether the breast condition resolved following the substitution.

EFV was the ART drug most often targeted for change when a breast abnormality occurred (n=16/19, 84.2%), followed by D4T (n=5/19, 26.3%). In this population, EFV was most often substituted for NVP (n=9, 47.3%) followed by LPV/r and ATV/r (n=2/41, 10.5%). Three patients received more than one drug substitution or cessation simultaneously. The only three cases of resolution of the breast condition following ART substitution were observed, with all three cases occurring in the EFV to NVP group. No association was found between the resolution of breast conditions and substitution with NVP (p=0.221). For all other cases, there was no recording of resolution of the breast abnormality (n=16, 84.2%)

Table 15: Details of ART substitution and whether resolution took place (n=19)

Substitution	Count (%)	Resolved*
EFV to NVP	9 (47.3)	3
EFV to LPV/r	2 (10.5)	0
EFV to ATV/r	2 (10.5)	0
D4T to ABC	3 (15.8)	0
EFV to LPV/r and D4T to TDF	1 (5.3)	0
EFV to NVP, D4T-DDI to ABC	1 (5.3)	0
ABC-3TC-EFV to LM	1 (5.3)	0

** Implies resolved prior to 31 December 2014 (end of the study period)*

4. Discussion

The clinics that were studied have large populations of adolescents receiving antiretroviral therapy with just under half of the files screened being those of adolescents, as opposed to children, receiving ART. With such large populations of patients, common side effects experienced in a particular age group become evident.

One such condition is breast abnormalities in those receiving ART. The prevalence of breast abnormalities in this cohort of HIV infected adolescents on ART was 5.9%. In male patients the prevalence was 7.9% which exceeds the background incidence of physiological gynaecomastia in adolescent males of 4%.[5]

Despite there being more girls included in the study, there were significantly more breast events seen in male patients. Breast abnormalities in males are easier to detect and define due to the "all or nothing" nature of the abnormalities; that is male patients are not expected to develop breasts and so, when they do, it is easily noticeable. In girls, breast development includes a spectrum of changes, which makes an abnormal event more difficult to define.[27,4]

A significant difference in age was observed and adolescents with breast abnormalities were older than those without breast conditions. This suggests that most abnormalities of the breast in HIV-infected adolescents on ART are experienced in mid-adolescence, at a median age of 15.5 years, which is somewhat later than physiological gynaecomastia in boys, which is seen to peak earlier between the ages of 13 and 14 years.[5]

The link between ART drugs and breast abnormalities has not been well established with different drugs and mechanisms being hypothesised for the development of breast conditions in HIV-infected individuals.

Various drugs such as D4T and AZT have well documented links to lipodystrophy and may in turn affect the development of breasts through the same mechanism.[9] However other drugs, such as EFV, have not been shown to be a significant cause lipodystrophy and yet have been postulated to cause breast conditions.[8,13] This

suggests that there is more than one possible aetiology interacting to result in a similar outcome of abnormal breast conditions in patients receiving ART.

In this cohort of patients, most were receiving an NNRTI as opposed to a PI-based regimen which is expected in accordance with the recommended South African ART guidelines for first line ART.[18] The most frequently used NNRTI was EFV and this was combined with ABC and 3TC, which is the recommended first line regimen in South Africa for children and adolescents 3 to 14 years of age, in 65.2% of cases.[18] ABC was not shown to be associated with abnormal breast development (p=0.363). This suggests that in the majority of patients who were receiving ABC/3TC/EFV, EFV remains the likely candidate for causing breast abnormalities.

LM was the second most popular choice for adolescents in this cohort, with 11.6% of patients receiving this monotherapy regimen. LM is used in those children and adolescents who have adherence difficulties on their first line regimen and are unable, due to social circumstances, to take a more demanding second line regimen.[24] While patients on LM did experience abnormalities of their breasts, the prevalence of abnormal breast conditions was lower than that of the rest of the cohort making lamivudine an unlikely candidate for causing this phenomenon.

Patients on PIs are likely to be receiving a second line regimen after having failed a first line regimen with over 10% of included patients being on a PI-based regimen. With the advent of ART for children in the public sector in South Africa only occurring in 2004,[22] relatively few individuals in this cohort would have been under three years of age at the time and therefore eligible for a PI-based regimen as their first line.[22] Indeed, the median age of initiation for both groups of patients in this cohort was over 8 years of age which suggests they were most likely started on an NNRTI as

their first line regimen in accordance with the guidelines at the time.[22] This seemingly older age of ART initiation in vertically infected children could be explained the rollout of ART only taking place in South Africa in 2004 and the relative complexity of the childhood ART guidelines as well as difficulties of ART accessibility at the time.[22]

Almost a third of this cohort was treatment experienced having received three or more ART regimens. They therefore would have had an exposure to a variety of ARV drugs. However, it was reassuring that while many patients were treatment experienced, three quarters of patients were receiving an NNRTI and were, therefore, still effectively on first line ART.

At present there are four plausible mechanisms by which ARV drugs cause breast abnormalities: 1) oestrogen receptor activation by ARV drugs particularly EFV,[6,10,13] 2) immune reconstitution inflammatory syndrome (IRIS),[8] 3) lipodystrophy syndrome[9] and 4) hypogonadism.[16]

4.1. Oestrogen receptor activation by EFV

At the time of reporting of an abnormal breast event, receiving EFV was significantly associated with the development of breast abnormalities in this cohort of patients. Furthermore, all patients with abnormal breast conditions had received EFV as part of a previous regimen and their exposure was often long-term with the mean time of exposure being 5.5 years. EFV use was associated with the development of abnormal breast conditions in this adolescent population which is supported by other adolescent and adult studies with similar outcomes.[1,6,10,13]

Resolution of abnormal breast conditions only occurred in three patients and all of these patients had been changed off EFV and started on NVP. This drug substitution

was the most commonly observed and other substitutions of EFV or other ART drugs occurred in numbers too small to show a trend. The failure to report that an abnormality resolved in the rest of those suffering from breast abnormalities led to uncertainty about whether other drug substitutions were effective. At least two patients were later referred to a specialised breast clinic suggesting no resolution. Further follow up of the affected patients may provide more answers as to whether the interventions were effective.

4.2. IRIS

The hypothesised mechanism of breast enlargement in IRIS is thought to be through an increased production of cytokines which increase breast tissue aromatase activity causing increased oestrogen production.[8] Once full immune reconstitution has taken place, the levels of these cytokines fall, which should lead to full resolution.

The median time for receiving ART in this adolescent cohort was around five years and the median duration of their current ART regimen was two years. The majority of patients had a CD_4 count greater than 500 at the time of their breast event. IRIS usually occurs in patients with lower baseline CD_4 counts particularly those below 50 cells/µl.[15]

Unlike in adults, the proximity of ART initiation to breast enlargement is often not as pronounced in adolescents. Adults appear to develop gynaecomastia within months of starting antiretroviral therapy,[6] whereas the timing is less predictable in adolescents with most breast abnormalities occurring after years of treatment with the same regimen. This suggests that IRIS, which occurs within three months of starting ART, is unlikely to be the mechanism of abnormal breast formation in adolescents.[14]

4.3. Lipodystrophy

Lipodystrophy was by far the most common comorbidity seen in patients with breast abnormalities and has been attributed to be the cause of some cases of breast enlargement in adults on ART.[9] Nearly half of those with abnormal breasts were reported to have comorbid lipodystrophy with fat accumulation being the most frequently reported feature followed by lipoatrophy. Lipodystrophy is often associated with D4T and AZT, however few patients in this study were on D4T- or AZT-containing regimens at the time of censure and very few breast events were documented while patients received regimens containing these drugs. Despite this almost 60% had received D4T as part of a previous regimen and had received the drug for a median duration of 4.9yrs (IQR: 1.8-7.2). D4T was found to be associated with the development of abnormal breasts (p=0.028), however the mechanism is most likely to be through fat accumulation as part of lipodystrophy. As only one patient was investigated by sonography, it is difficult to establish whether the breast abnormalities recorded are part of the spectrum of lipodystrophic changes, which may be mild and difficult to distinguish in their early stages, or isolated breast enlargement. Sonography is not available at primary healthcare facilities in South Africa and this may account for why very few patients were investigated using sonography (4.8%) and yet many more (51.2%) received an intervention for their breast condition.

4.4. Hypogonadism

The hypothesis in a case control study performed on a Spanish adult cohort was that that free testosterone levels in male patients with gynaecomastia on ART were significantly lower than a control group without breast abnormalities.[13] None of the male patients in our adolescent cohort had free testosterone levels performed to rule

out hypogonadism as a cause for their breast abnormality. Although there was a comment made in one patient's file regarding him receiving hormonal injections for hypogonadism, there were no other details provided as this condition was managed at another centre. As such it is difficult to draw any conclusions regarding hypogonadism as a modality causing breast abnormality in this cohort.

4.5. Adherence to Antiretroviral Therapy

Adherence to a prescribed treatment is generally difficult to assess. This is because patient / caregiver self-report is often the only modality available making measurement of adherence highly subjective. In many cases, self-report is strengthened by other objective measures such as the patient's viral load, clinic attendance records and pill counts. Good adherence to ART was reported by 63.4% of patients with abnormal breast conditions. This is supported by rates of virological suppression defined as less than 50 copies/ml in an excess of 70% in this population subset. This suggests that good adherence to ART, which can be clinically verified, may be a contributory factor in the development of abnormal breast conditions in adolescents. A limitation was that there were no comparative HIV-1 viral load results collected from those without breast condition to determine whether virological suppression is associated with breast abnormalities.

A positive association between EFV and breast abnormalities however was observed and it may be postulated that a dose-dependent relationship, that is as increasing amounts of EFV are ingested more breast abnormalities may be observed, should be investigated further in a prospective case control study.

Five patients were reported to have been lost to follow up around the time of breast abnormality. They may have become aware, through their clinician, that the

abnormalities of their breasts could have been caused by their ART and had decided to stop medication of their own accord. The role of this potentially dire effect on treatment adherence needs further investigation.

4.6. Breast abnormalities

Nearly half of the cases of breast abnormality were described somewhat vaguely as enlargement and just less than half were described using the terms gynaecomastia and lipomastia, which appear to be used interchangeably. The prevalence of severe breast abnormalities for the whole population was 0.5% which was the same as the CHIPS cohort of child and adolescent patients on ART in the UK.[1] Overall there appears to be a degree of indecision as to what is considered abnormal and what is considered part of the spectrum of normal adolescent breast development. Breasts could be described as abnormal by the clinician in a consultation to be later dismissed as normal by another. Below is an example from a patient file in the study:

"1st consultation: *Possibly developing early gynaecomastia - watch* (Doctor X)

2nd consultation: *Concern(ed) previously about developing gynaecomastia but looks fine today* (Doctor Y)

3rd consultation: *Gynaecomastia improving* (Doctor X)"

The grade of severity may also make it difficult to determine whether a change is normal or pathological with earlier cases frequently being confused with normal development. More severe conditions would be obvious and easier to grade. Although only two cases of abnormal breast development were detected early, it is likely that there were more early cases that were classed as normal or not detected. Cases of unilateral breast development would also be more easily classed as

abnormal compared to bilateral breast conditions which may be classed as normal breast development.

The severity of the breast condition was often disputed between clinicians with some choosing to intervene in a condition and others feeling the intervention would be more cumbersome to the patient than the distress caused by the condition. An example of this would be changing patient to a regimen with a high pill burden or increased medication frequency, which would adversely impact on adherence. Other difficulties with the classification of breast abnormalities include the patient's and clinician's own beliefs of the severity of a breast condition. A patient's attitude towards their breast condition, which may be based on the opinions of his family and friends, would influence his likelihood of reporting a suspected abnormality to a clinician. A patient may also be overly aware of a seemingly mild manifestation of gynaecomastia and request intervention even when the clinician feels that it is unwarranted. Similarly, a clinician may not prescribe an intervention, despite them believing the patient's breast condition to be abnormal, if the patient does not feel that this is necessary.

Obtaining a definitive diagnosis of gynaecomastia or breast enlargement remains a challenge through the lack of available diagnostic modalities and expertise. Ultrasonography is able to assist with determining whether there is a glandular or fatty predominance, however this is not definitive.[8] Coupled with the difficulty of obtaining an ultrasound, performed by a trained technician in the South African Primary Healthcare setting, this was not fully utilised as a modality to assist with appropriately managing patients with abnormal breast conditions.

There are classification systems used to grade the severity of gynaecomastia in male patients described by Simon et al[28] and Rohrich et al[29], however such systems exclude the detection of abnormalities in female patients. These examples highlight the need for an official and validated classification of the disorders of the breast seen in adolescents on ART to assist with diagnosis and management of such conditions.

4.7. Interventions for breast abnormalities

While over half of patients received an intervention (n=21/41), only three cases were reported to resolve. Of these patients, all three received an EFV to NVP substitution whereas no resolution was seen in patients with any other drug substitution, drug prescription or prescribed lifestyle changes. There was no association established between the resolution of abnormal breast events with substitution to NVP however this is most likely due to the small numbers of breast abnormalities and the limited study period.

While Tamoxifen, an anti-oestrogen drug used for treating gynaecomastia,[5,10] was prescribed for one patient, the clinic did not stock the drug and thus it was never received by the patient. For this reason, Tamoxifen remains an unexplored intervention in this cohort.

Most patients' breast abnormalities were not reported to resolve and very few were referred on to the next level of care. Three patients were referred to a specialised breast clinic where the recommendation was made for one female patient that continued observation of her breast size should be performed until the end of puberty. None of the three patients received a definitive intervention. This could be attributed to a lack of knowledge on how to manage these cases at a specialist level and further study is needed to develop the best treatment management algorithms.

4.8. Comorbidities and concomitant medication

There are several proposed conditions that may cause pathological gynaecomastia in adolescents and these include obesity, adrenal and testicular neoplasms, Klinefelter syndrome, Peutz-Jeghers syndrome, thyrotoxicosis, cirrhosis, primary hypogonadism, congenital adrenal hyperplasia, androgen insensitivity, malnutrition and particular medicines.[5] The proposed mechanism of causality in adolescents is oestrogen excess which is able to activate oestrogen receptors in breast tissue.[10] One or more comorbidity was observed in almost three-quarters of patients with abnormal breast conditions. Lipodystrophy was noted in just under half of the patients.

One patient was recorded to be obese and lifestyle interventions, for example diet and exercise, were suggested for that patient in order to improve their breast condition. However the relationship between gynaecomastia and obesity is not well established with there being conflicting reports.[5] Most patients (80%) had BMIs within the normal range or tending towards being underweight, with just under 20% tending to be overweight. This suggests that obesity as a cause of breast enlargement in this cohort is only a possible mechanism in less than a fifth of patients.

One other patient was reported to be receiving hormonal injections for hypogonadism. No investigations were performed for suspected other causes of breast abnormality in this study and this case stands in isolation.

Other concomitant medications may also contribute to the development of breast abnormalities with 6/10 patients having received one or more concomitant medication and over 4/10 receiving two or more concomitant medications. The high

levels of different drug exposures in this cohort may interfere with establishing a causal relationship between some drugs and breast development. For those drugs documented to cause gynaecomastia, only isoniazid, received by two patients, and hormonal injections, used by one patient, were seen in this cohort at the time of breast abnormality.[7]

5. Limitations

There was no opportunity, in this retrospective record review, to compare the frequency of breast abnormalities in this population with a similar control group (adolescents who are not HIV infected). Therefore, a background incidence of breast abnormalities in adolescents, particularly females, could not be established from which to perform a comparison. A prospective study will be needed to further investigate this.

Physical examination can be limited during patient visits in South African public health facilities providing ART. The onus is often on the patient to report their breast condition to the clinician before further enquiry will take place. This would have led to the reported incidence of breast abnormalities being lower than the actual incidence. As the issue of breast abnormalities in adolescents on ART becomes increasingly familiar to clinicians, there are likely to be more cases reported and referred for these issues. Therefore, a similar retrospective review a later year might reveal an increase in cases of breast conditions.

As this review did not extend to reviewing patient's records who were less than 10 years old in the study period (2014), a prevalence for breast abnormalities in patients less than 10 years old could not be commented on.

Little investigation took place into the cause of breast abnormalities in this cohort. The lack of availability of ultrasonography at primary healthcare level as well as the lack of the expertise required by a sonographer to adequately diagnose mild to moderate breast abnormalities and to distinguish between glandular and fatty breast predominance, would impact the diagnosis and, subsequent management, of these conditions. Blood tests were only performed in two patients and the results were thereafter not followed up. This is most likely due to a paucity of guidelines for clinicians to manage such conditions.

This record review was performed at three facilities among which the treating clinicians have substantial interaction and, therefore, may have similar algorithms for managing their patients. This may have affected the detection of cases, descriptions used; paucity of investigation as well as interventions used particularly those of drug substitution.

6. Conclusion

Children may be started on ART and could, after many years and only once they are in mid-adolescence, develop breast abnormalities. This discounts an IRIS phenomenon as a likely cause of abnormal breast development in adolescents. The timing however also does not correspond with the incidence of physiological gynaecomastia observed in male patients.[5] While lipodystrophy was a common comorbidity in those with breast abnormalities, sonography was not routinely available to establish a definitive breast diagnosis. Similarly, the role of hypogonadism was not fully explored.

Being adherent to one's ART regimen seemingly places patients at an increased risk of developing breast abnormalities. This negative result of good adherence to ART, in particular, needs to be consistently addressed by clinicians managing adolescents on ART, as it may lead to non-adherence in those patients previously taking their antiretrovirals correctly.

It is proposed that the drug choice for adolescent ART, particularly those regimens containing EFV, when interacting with the fluctuating hormonal levels during puberty, may account for this unique entity of breast abnormality. The role of EFV in the development of breast abnormalities in both male and female adolescents requires further exploration to determine the appropriate investigations to perform and the most effective interventions to employ.

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Appendix 1: Ethics Clearance Certificate

R14/49 Dr Jackie Dunlop and Prof Cynthia Firnhaber et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M141134

NAME: (Principal Investigator)	Dr Jackie Dunlop and Prof Cynthia Firnhaber et al
DEPARTMENT:	Clinical Medicine Alexandra Health Centre and Univerisity Clinic Right to Care Paediatric Clinic Witkoppen Health and Welfare Clinic
PROJECT TITLE:	HIV-Infected Adolescents on Anti-Retroviral Therapy: A Retropective Descriptive Cohort Study of Breast Abnormalities Documented during Routine Care
DATE CONSIDERED:	28/11/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	
APPROVED BY:	Professor PE Cleaton-Jones Chairperson, HREC (Medical)
DATE OF APPROVAL:	09/12/2014

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

28/6/17.

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