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Subjective Clinical Lipoatrophy Assessment Correlates with DEXA-measured limb fat

Marisa Tungsiripat, MD¹, Mary Ann O’Riordan, MS², Norma Storer, RN³, Danielle Harrill³, Jason Ganz, MD⁴, Daniel Libutti, BS⁵, Mariana Gerschenson, PhD⁵, and Grace A McComsey, MD^{2,3,4}

¹Department of Infectious Diseases, Cleveland Clinic Foundation, Cleveland, OH

²Department of Pediatrics, Rainbow Babies and Childrens’ Hospital, Case Western Reserve University, Cleveland, OH

³Rainbow Babies and Childrens’ Hospital and Case Western Reserve University, Cleveland, OH

⁴University Hospitals Case Medical Center, Cleveland, OH

⁵Department of Medicine, Hawaii AIDS Clinical Research Program, University of Hawaii, Honolulu, HI

Abstract

Objectives—Although physician and patient-rated diagnosis of lipoatrophy are currently used as a basis for inclusion into clinical trials, few studies have compared physician or patient-rated lipoatrophy severity with objective measures. We aim to assess the validity of physician- and patient-rated diagnosis of lipoatrophy by evaluating the correlation between clinical assessments of lipoatrophy and objective fat indices.

Methods—This cross-sectional study evaluated the association between clinical lipoatrophy scores and DEXA-measured limb fat (n=154) and subcutaneous fat mitochondrial DNA levels (n=80) in HIV+ individuals.

Results—There was a significant negative correlation between DEXA-measured limb fat and lipoatrophy scores generated by either the patients (r= -0.27, p=0.008) or the physician (r=-0.48, p<0.0001). Also, a significant positive correlation was found between the patient-generated lipoatrophy score and the physician score (r=0.68, p<0.0001). However, there was no correlation between fat mtDNA levels and DEXA-measured limb fat (r= -0.09, p=0.42) nor with physician or patient-generated lipoatrophy scores (r=-0.09; p=0.43 and r=0.04; p=0.71, respectively.)

Conclusion—These results suggest that physician and patient-rated lipoatrophy scores may be useful surrogates for more expensive measures of lipoatrophy, which could be reserved for research studies.

Keywords

Lipoatrophy; Lipodystrophy; DEXA

Introduction

The metabolic complication lipoatrophy remains prevalent and a cause of significant morbidity for individuals living with HIV.¹ Lipoatrophy is subcutaneous fat wasting of the face, arms, buttocks, and/or legs and has been associated with thymidine nucleoside reverse transcriptase inhibitor (NRTI) agents.¹⁻⁴ Lipoatrophy is frequently distressing to patients from a cosmetic standpoint alone. Additionally, it is often stigmatizing as a prominent and visible association with HIV/AIDS. Lipoatrophy has been shown to decrease the quality of life of HIV-infected individuals and adherence to antiretroviral therapy (ART).^{5, 6}

No treatment options are currently available for patients with lipoatrophy who have already switched off of thymidine analogs. Although the development of lipoatrophy is expected to be less common with newer antiretroviral regimens, thymidine analogs remain in wide-spread use internationally and in salvage regimens in developed countries. Thus, clinical trials evaluating treatment options for lipoatrophy remain ongoing.

The diagnosis of lipoatrophy for both purposes of patient care and of determining inclusion into most HIV clinical trials has been typically with patient self-report and/or physician confirmation of the diagnosis.^{4, 7} In many HIV clinical trials, dual-energy x-ray absorptiometry (DEXA) has been used to assess longitudinal changes in fat after interventions,^{4, 7-9} since DEXA can reliably quantify changes in regional fat mass.^{10, 11} The quantification of mitochondrial DNA (mtDNA) content in peripheral blood mononuclear cells (PBMC) and subcutaneous adipose tissue obtained by fat biopsy has been correlated with the degree of lipoatrophy associated with ART.^{12, 13} Nucleoside reverse transcriptase inhibitors (NRTIs) inhibit reverse transcriptase and also DNA polymerase γ which essential to mitochondrial (mt) DNA replication, leading to its depletion and oxidative phosphorylation disorders in multiple tissues including fat. In terms of PBMC mtDNA, the measurements have correlated with lipoatrophy in some studies but not in others.^{12, 14} However, DEXA scans and fat biopsies are not routinely performed to determine eligibility for inclusion into clinical trials. For study screening purposes, DEXA and fat biopsy would be cost prohibitive and could not be justified from a safety standpoint.

Although physician and patient-rated diagnosis of lipoatrophy are presently commonly used as a basis for study entry, few studies have compared these physician or patient-rated subjective measures of lipoatrophy with objective measures.^{15, 16} We aim to examine the validity of physician- and patient-rated subjective diagnosis of lipoatrophy by evaluating the correlation between scores derived from simple, easy-to-use questionnaires and DEXA-measured limb fat and fat mtDNA levels.

Methods

Patients participating in this cross-sectional study are those who presented to the Case Western Reserve University HIV Metabolic Center or to the Cleveland Clinic Foundation for evaluations for inclusion into metabolic clinical trials. Inclusion criteria were HIV infection, age 18 years or older, and either 1) ART-naïve or 2) receiving stable ART for at least 6 months and have a clinical diagnosis of lipoatrophy. Clinical lipoatrophy has been defined as physician and patient self report of fat loss in at least 2 of the following areas: face, arms, legs, and buttocks. Exclusion criteria for this study include current opportunistic infections, renal or hepatic impairment, coagulopathy, abnormal PT/PT, diagnosis of diabetes, active endocrine disorders, and history of recent use of hormonal therapies.

Patients were enrolled from May 2005 to December 2007. They were enrolled at the Case Western Reserve University HIV Metabolic Center or at the Cleveland Clinic Foundation in

Cleveland, OH. The Institutional Review Board (IRB) Committees approved the study. All patients gave written informed consent.

Study evaluation included clinical examinations, blood testing, clinical lipoatrophy questionnaires, whole body DEXA scanning for body fat composition and fat biopsy. The blood tests were done in a fasting state (at least 8 hours) and included lipid panel, insulin, glucose, and PBMC for measurements of mtDNA levels.

Clinical lipoatrophy scores were obtained by questionnaires obtained at study entry. These questionnaires rated the loss of fat in predefined body areas. The lipoatrophy score was based on questions about fat loss in the arms, legs, buttocks and the face. Assessments within each of these sites were rated “0” for “absent”, “1” for “mild”, “2” for “moderate”, and “3” for “severe.” Thus, the lipoatrophy score could vary between 0-12. The same questionnaire was completed separately and independently by the patient and the physician.

The DEXA scans were performed at a single site (Case) on all study subjects, using a Hologic QDR-4500A (Hologic Inc, Bedford, MA). The DEXA were assessed with a dedicated scanner and technologist. The subcutaneous fat tissues were collected from the lower abdomen by excisional biopsies performed under local anesthesia by an experienced plastic surgeon (JG). DNA was extracted from subcutaneous adipose tissue biopsies (stored at -70°C in RNA later) or Peripheral Blood Mononuclear Cells (PBMCs viably frozen) using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Inc., USA). Total DNA was quantified in ng/μl using a UV-Vis ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) and integrity examined by agarose gel electrophoresis. Analysis of mtDNA copies/cell was conducted by absolute quantitative real-time PCR as previously described.^{12, 17}

Statistical analysis was performed with Spearman rank correlation coefficients which were used to describe the relationship between two continuous variables. The level of significance was set at 0.05 for all analyses. All analyses were carried out using SAS, v. 8.2 (The SAS Institute, Carey, NC)

Results

Overall, 154 HIV-infected subjects were enrolled. Eleven (7%) of the subjects were naïve to ART and 143 (92.8%) were on stable ART and referred for lipoatrophy clinical trials. Demographic and baseline characteristics are outlined in table 1. Twenty-nine percent were female; 46% were Caucasian. The median (range) age was 47 (25-73) years, and the median BMI was 26 (11-41) kg/m².

Of the 143 subjects who were receiving ART, the median (range) duration at the time of study entry of NRTI therapy was 91 (3-327) months, and the median duration of thymidine NRTI therapy was 64 (0-327) months. The median (range) duration of NNRTI and PI therapy was 10 (0-302) and 42 (0-200) months, respectively.

The median (range) lipoatrophy scores generated by the patients and physicians were 6 (0-12) and 7 (0-12), respectively. The median (range) DEXA-measured limb fat was 5158 (2129-20,806) grams. The median (range) limb fat values tended to be lower in the subjects on ART with clinical lipoatrophy than ART-naïve subjects, 5005 (2129-20,806) and 7661 (2268-18,313) grams, respectively. However, this difference did not reach statistical significance (p=0.08).

In the subset of 80 subjects who had fat biopsies as part of separate metabolic studies, the median (range) fat mtDNA was 496 (60-3091) copies/ cell. The median (range) fat mtDNA levels were significantly lower in ART-treated subjects compared to levels in untreated

subjects: 395 (60-3091) and 1062 (485-2721), respectively ($p=0.002$). Of these, in the 79 subjects with PBMC mtDNA levels, the median (range) values were 221 (10-587) copies / cell. Levels were similar in ART-treated and -untreated groups.

There was a significant negative correlation between DEXA-measured limb fat and lipoatrophy scores generated by both the patient ($r= -0.27$, $p=0.0008$) and the physician ($r=-0.49$, $p<0.0001$). In addition, there was a significant positive correlation between the patient-generated and the physician-generated lipoatrophy score ($r=0.68$, $p<0.0001$). See Figures 1 and 2.

There was a significant correlation between PBMC mtDNA levels and fat mtDNA levels ($r=0.43$, $p<0.0001$). However, there was no correlation between fat mtDNA levels and DEXA-measured limb fat ($r= -0.09$, $p=0.42$) nor with patient or physician-generated lipoatrophy scores ($r= -0.09$; $p=0.43$ and $r= -0.04$; $p=0.71$, respectively). These observations held true in both ART-treated and -untreated subgroups when these were analyzed separately. Similarly, there was no significant correlation between PBMC mtDNA levels and DEXA-measured limb fat ($r= -0.045$, $p=0.69$) nor with patient -generated lipoatrophy scores ($r= 0.18$, $p=0.11$). However, PBMC mtDNA levels did correlate with physician generated lipoatrophy scores ($r=0.25$, $p=0.026$).

When considering separately the ART-naïve subjects, there was no significant correlation between the DEXA-measured limb fat and patient or physician-generated scores. However, in considering only ART-treated subjects, a significant correlation was found between DEXA-measured limb fat and patient and physician-generated scores ($r= -0.22$, $p=0.0072$ and $r= -0.51$, and <0.0001 , respectively).

Discussion

A number of studies have examined subjective and objective assessments for facial lipoatrophy.^{18, 19} However, few studies have examined these subjective and objective assessments for body fat changes.^{15, 16, 20} Additionally, the focus of these prior studies have differed from our present study. In our study, we chose to examine the validity of clinical assessments of lipoatrophy scores independently generated by patients and physicians with objective assessments of lipoatrophy.

In this study, we showed that both patient- and physician-generated clinical lipoatrophy scores significantly correlated with DEXA-measured limb fat. This finding is consistent with a prior study¹⁵ which reported significant correlations between DEXA-measured limb fat and physician assessment and patient assessment ($r= -0.49$; $p<0.0001$ and $r=-0.48$; $p<0.0001$, respectively). In the prior study, the physician and patient assessments also correlated with a studied lipoatrophy case definition score ($r=0.64$, $p<0.0001$ and $r=0.60$, $p<0.0001$, respectively) which incorporated the parameters of: gender, age, duration and CDC category of HIV diagnosis; clinical data of waist-to-hip ratio; metabolic parameters of HDL-cholesterol and anion gap; CT-measured visceral abdominal fat: total abdominal fat (VAT:SAT) ratio, and DEXA-measured trunk, limb and leg fat percent. Although correlations between the studied case definition score and the subjective lipoatrophy assessments may have been stronger, this studied case definition score requires both CT and DEXA measurements which are often not practical and feasible for study entry or clinical settings.

Another study evaluated lipoatrophy and lipohypertrophy changes (defined as concordance between participant report of change and examination) with MRI. Differing from our study, the focus of this study was assessing which fat changes were HIV-specific in comparison with control subjects. HIV-infected men with clinical lipoatrophy had less subcutaneous adipose tissue in peripheral sites than HIV-infected men without peripheral lipoatrophy.²⁰

A recently presented study reported that 30% of DEXA-measured limb fat must be lost before occurred before fat loss was clinically evident by patients and/or physicians ¹⁶. The study by Podzamczar was remarkably small (n=54) for the stated purpose. In addition, this relative lack of subjective/ objective correlation may have been related to that “mild fat loss” and “no fat loss” groups were considered together as a homogeneous group of non-clinically evident lipoatrophy. In contrast, our present study correlates clinical fat changes with DEXA measurements across a spectrum of body fat. We found that the correlation between clinical lipoatrophy scores and the objective DEXA-measured limb fat in our study was particularly strong in the subset of patients on ART therapy. This finding would similarly suggest that fat distribution may be difficult to assess in ART-naïve patients by patients and/or physicians.

Neither the clinical lipoatrophy scores nor DEXA-measured limb fat consistently correlate with fat mtDNA levels. Similarly the PBMC mtDNA levels did not significantly correlate with either subjective or objective measures. This lack of correlation seen with PBMC mtDNA levels is consistent with a prior study. ²¹ However, the authors of this relatively small study (n=24) did note a correlation between clinical lipoatrophy and fat mtDNA ^{21, 22} which differs from our findings in our present study. Consistent with our results, a study evaluating an intervention in subjects with established lipoatrophy reported changes in lipoatrophy scores over time, but saw no changes in fat or PBMC mtDNA over this time. ²² The lack of correlation seen may be related to the relatively short duration of the prior study (16 weeks) or the cross-sectional nature of this present study.

Conclusion

In summary, both patient and physician-generated clinical lipoatrophy scores correlated with DEXA-measured limb fat. This correlation was particularly strong in the subset of patients treated with ART with established lipoatrophy. These results suggest that physician- and patient-rated lipoatrophy scores may be useful surrogates for more expensive measures of lipoatrophy.

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Figure 1.
Correlations between subjective physician lipoatrophy score and DEXA-measured limb fat

Figure 2.
Correlations between subjective patient lipoatrophy score and DEXA-measured limb fat

Table 1

Baseline characteristics of all study subjects (n=154)

	N(%) or median (range)
Age (years)	47 (25-73)
Sex (male)	125 (81%)
Race:	
White	71 (46%)
African American	65 (42%)
Other	18 (12%)
BMI (kg/m ²)	25 (11-41)
Duration of HIV (years)	11 (0.1- 25)
Duration of NRTI therapy (months) n=143	91 (3-327)
Duration of thymidine NRTI therapy (months): n=143	64 (0-327)
Duration of NNRTI therapy (months) n=143	10 (0-302)
Duration of PI therapy (months) N=143	43 (0-200)
CD4+ cell count (cells/mm ³)	569 (67-1819)
HIV-1 RNA < 50 copies/ mL	97 (68%)