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PRECLINICAL STUDY

Differential effects of tamoxifen and anastrozole on optic cup size in breast cancer survivors

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Abstract
Introduction The main purpose of this study was to determine whether the optic cups of tamoxifen users and anastrozole users differ in size, with the cups of the tamoxifen users being smaller.
Methods Optic nerve head (ONH) topography was measured using a commercially available, confocal scanning laser ophthalmoscope for three populations of amenorrheic women ages 40–69 years: subjects using (1) tamoxifen (20 mg/day) or (2) anastrozole (1 mg/day) for ≤ 2 years as adjuvant therapy after successful primary treatment for breast cancer, and (3) control subjects with no breast cancer histories and not using any hormonal medication. All subjects had excellent visual acuity and healthy eyes, based on conventional photographic assessment.
Results The cup volumes of the tamoxifen users were shown to be significantly smaller than the cup volumes of the anastrozole users, which were indistinguishable from normal. Because the cup volumes of the tamoxifen users decreased markedly with age at about 50 years and because anastrozole is indicated only for post-menopausal women, comparisons were reassessed for subjects older than 50 years. For these subjects, the cup volumes of the tamoxifen users averaged less than half of the volumes for each of the other two subject groups, and significant between-group differences existed in both the lateral (cup area) and axial (cup depth) directions. In contrast, any between-group differences at the ONH margin were small and not significant.
Conclusions The results of this study suggest that the ONH be assessed biomorphometrically for tamoxifen users reporting visual change that cannot be attributed to non-tamoxifen causes. The ability of modern intraocular imaging techniques to reveal anatomic change on the order of tens of microns may be useful for assessing tamoxifen-induced effects occurring simultaneously elsewhere in the brain, particularly since the presence of small cups is consistent with the possibility of tamoxifen-induced astrocytic swelling.

Keywords Adjuvant endocrine therapy · Anastrozole · Breast cancer · Eye · Optic cup · Optic nerve head · Tamoxifen

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Introduction

Selective estrogen receptor modulators (SERMS) and aromatase inhibitors are the two major classes of medications used in the United States as adjuvant endocrine therapy for early-stage, hormone-receptor-positive breast cancer. Of the SERMS that are FDA-approved for this purpose [1], tamoxifen has been the most widely used, and for several decades it has been

prescribed extensively for women of all ages [2]. The first aromatase inhibitor to be FDA-approved for early-stage breast cancer was anastrozole (Arimidex®), in 2002 [2]. However, because aromatase inhibitors do not block estrogen production adequately for pre-menopausal women [3], they are indicated for use by post-menopausal women only [4], for whom estrogen production is entirely non-ovarian [5]. Initial reports indicate that anastrozole is more effective than tamoxifen at preventing cancer recurrence [6, 7]. Thus, anastrozole now is being recommended for many women who would have been prescribed tamoxifen several years ago [8]. Because the use of aromatase inhibitors is likely to continue to increase and because the use of tamoxifen may become progressively more restricted to younger women, the potential side effects of anastrozole need to be evaluated, and the influence of age on tamoxifen side-effects needs to be closely examined.

Of all the documented or putative effects of tamoxifen on the visual system [9], tamoxifen retinopathy may be the most widely known. However, tamoxifen retinopathy is uncommon [10] and possibly cumulative-dose-dependent [11]. In contrast, Eisner et al. [12] found that short-term tamoxifen users (i.e., ≤2 years of use) often have smaller optic cups than age-matched female control subjects, in a study of women who had been selected for the absence of any overt eye disease. This result was consistent with the possibility that tamoxifen often causes a subclinical degree of swelling at the optic cup, particularly since tamoxifen is a potent blocker (at least in culture) of swelling-activated chloride channels [13, 14], such as those in astrocytes [15, 16], and astrocytes are the predominant glial cell in the cup [17, 18]. If tamoxifen indeed causes optic-cup swelling via its ability to block swelling-activated chloride channels, the cup sizes of anastrozole users would be expected to more closely resemble those of female control subjects than those of tamoxifen users. The present study tested this prediction.

Because of changing trends in adjuvant breast cancer treatment, the present study also assessed the effect of age on optic nerve head (ONH) parameters. The results support the inference that tamoxifen usage can lead to small cups, although apparently mainly for women older than about 50 years. All measurements for this study were obtained using confocal scanning laser ophthalmoscopy with the Heidelberg Retina Tomograph II (HRT). This commercial instrument was introduced in 1999 for routine clinical use, but until now, it has been used mainly to assess glaucoma and related conditions.

Methods

Subjects

Three groups of amenorrheic women ages 40–69 years old were recruited for this study: (1) women using the standard dose of 20 mg tamoxifen daily as adjuvant therapy for breast cancer for at least 4 months but no longer than 2 years, (2) women using the standard dose of 1 mg anastrozole daily as adjuvant therapy for breast cancer for at least 4 months but no longer than 2 years, and (3) women not using any hormonally acting medications. This third group served as an approximately age-matched control group. The 2-year duration-of-use requirement was based on results from several studies of vision [19, 20] and the eye [12] indicating that short- versus long-term tamoxifen use can be distinguished operationally using a 2-year cut-off. The 4-month duration-of-use requirement was included to help ensure that the medications had time to act and that the body's response had a chance to stabilize [21, 22].

All tamoxifen and anastrozole users had completed primary treatment for breast cancer, and all were fully active and able to perform their daily pre-cancer activities without restriction. None of the control subjects had positive breast cancer histories or had previously used breast cancer medication for any purpose. Similarly, none of the tamoxifen users had previously used a hormonally acting breast cancer medication other than tamoxifen, and none of the anastrozole users had previously used a hormonally acting breast cancer medication other than anastrozole. In addition, none of the subjects in any group had ever used the SERM raloxifene.

With one exception, all analyses for this report are based on data from subjects who had been amenorrheic for at least 6 months at the time of testing. The one exception is an analysis comparing data from control subjects with data from an additional group of women (the fourth subject group overall) who met the same eligibility criteria as the control group, except for the absence of menses.

All subjects, regardless of group, met a rigorous set of eligibility criteria for excellent ocular health in order to reduce the roles of confounding factors that could complicate the data. These are the same criteria we have used previously for several types of studies [12, 19, 23]. These criteria are: (1) 20/20 or better visual acuity in one eye and 20/25 or better visual acuity in the other eye, (2) no evidence or suspicion of eye disease on undilated direct ophthalmoscopic examination and on subsequent evaluation of individuals'

stereoscopic color fundus and ONH photographs by a glaucoma specialist (author JRS) who was masked from identifying subject information, (3) no history of eye disease or ocular hypertension, (4) no diabetes, (5) intraocular pressure (IOP) ≤22 mmHg on Goldmann applanation and no between-eye IOP difference >2 mm Hg, (6) no myopia >5 diopters, (7) no use of any medication (other than tamoxifen) known to affect vision, (8) no history of ocular surgery, and (9) normal color vision (i.e., no worse than a single minor transposition error on the D-15 test conducted under Macbeth illumination). There was no suggestion of papilledema for any subject (as for criterion #2).

Demographic data for eligible subjects with usable HRT data (see second paragraph of Procedures) are given in Table 1 for each of the three amenorrheic subject groups plus the additional group comprised of women without breast cancer histories and still experiencing menses. The mean ages of the tamoxifen users were slightly lower than the mean ages of the anastrozole users and amenorrheic control subjects possibly because some tamoxifen users were not menopausal despite being amenorrheic [24]. For reference, the median age of natural menopause in American society is 51 years [25].

All subjects were unpaid volunteers. Recruitment methods have been described previously [19]. All subjects were Caucasian, except for one Asian subject still experiencing menses. After being informed of the nature and possible consequences of the study, all subjects gave written informed consent to participate in this study. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the OHSU Institutional Review Board and the OHSU Cancer Institute. For each subject, HRT data and IOP measurements were obtained at the same testing session. Subject recruitment and assessment lasted several years, until usable HRT data had been collected for 20 eligible anastrozole users.

Procedures

ONH scans were obtained using the HRT II according to standard techniques, after which a contour line is drawn on the average of three rapid-succession scans to define the margin of the ONH (i.e., of the optic disk) [26]. Stereo color photographs of the ONH were used to assist with contour-line placement, and extensive use was made of the ability of the HRT II software to rotate images in three dimensions. Contour lines were drawn with the grader masked from all other subject information, including subject group and age. Additional details have been presented previously [12]. However, we had previously placed the contour line as close as possible to the dark-to-light color change in the HRT reflectance image in order to define the margin of the ONH [12], but for the present study we ensured that the contour line was placed on stable rim tissue on top of the ring of Elschnig [27], which is often most discernable from the underside of the 3D image, as a ridge circumscribing the ONH. With these 3D-based contour lines, the mean measured disk areas for each of our subject groups were very nearly centered within the norms provided by Heidelberg Engineering on the HRT II printout. Thus, with one exception, all analyses of HRT values are based on the contour lines drawn according to the more recent set of criteria, which are intended to place the contour line exactly at the disk margin, rather than slightly within it. The one exception was made to assess the importance (or lack of importance) of the precise contour line placement for documenting between-group

Table 1 Demographic data for each of the subject groups

	Amenorrheic subjects 40–69 years old			Amenorrheic subjects 51–69 years old			Not amenorrheic, no breast cancer history $n = 19$
	Anastrozole $n = 20$	Tamoxifen $n = 34$	Control, no breast cancer history $n = 32$	Anastrozole $n = 18$	Tamoxifen $n = 25$	Control, no breast cancer history $n = 29$	
Age (years)	58.2 (SD = 6.8)	54.5 (SD = 5.2)	57.2 (SD = 6.0)	59.6 (SD = 5.4)	56.5 (SD = 4.7)	58.2 (SD = 5.1)	48.0 (SD = 3.3)
IOP (mmHg)	15.7 (SD = 2.9)	15.5 (SD = 2.3)	15.6 (SD = 2.9)	15.7 (SD = 3.0)	15.5 (SD = 2.5)	15.7 (SD = 3.0)	15.5 (SD = 2.3)
Duration of medication use (years)	1.07 (SD = 0.45)	1.31 (SD = 0.49)	0	1.09 (SD = 0.47	1.36 (SD = 0.48)	0	0

IOPs are from subjects' test eyes only

differences. Thus, we conclude the Results section by briefly reporting the results of analyses based on data obtained using the earlier contour-line placement criteria.

Eyes were considered to have usable HRT data only if the average standard deviation for the height of each pixel in the 3 HRT scans did not exceed 50 μm, since average topographic standard deviation values above 50 μm are considered by Heidelberg Engineering to signify ''low image quality'' [28]. Image quality was equally good for each subject group. The mean average-topographic-standard-deviation for each of the three amenorrheic subject groups ranged from 20 to 22 (SD = 7–8) μm for all eligible subjects and also for subjects older than 50 years. Among women still experiencing menses, the mean was 23 (SD = 9) μm.

Although HRT scans were obtained for each subject's 2 eyes, only one eye per subject was used for data analysis for this paper. In this way, analyses could be limited to eyes with 20/20 or better acuity for a maximal number of subjects. Thus, test eyes were defined initially using the following steps applied in order as necessary: (a) the eye with the better acuity, even by 1 letter, than the fellow eye, (b) the eye with a lesser degree of spherical equivalent refractive error than the fellow eye, and (c) subject preference. In cases where the initially designated test eye had an HRT average topographic standard deviation value exceeding 50 μm, the fellow eye became the test eye for analysis purposes, but only if the fellow eye had 20/20 or better acuity and an HRT average topographic standard deviation value ≤50 μm. These are the same methods we used previously to designate test eyes [12].

Data analyses

Comparisons of central tendency across more than two subject groups were made using Kruskal–Wallis nonparametric analyses of variance (ANOVAS) because the distributions of some of the HRT indices were significantly non-Gaussian (1-sample Kolmogorov-Smirnov test) in ways consistent with the literature [29]. Similarly, post hoc comparisons between pairs of groups were made using Mann–Whitney U tests. Relations of HRT indices to age were evaluated for significance using Spearman rank-order coefficients because the strongest age trends were not linear. None of the reported *P*-values have been adjusted for multiple comparisons, but the P-values required for statistical significance of post-hoc tests as determined using a step-down Bonferroni procedure [30] are provided in the legend to Table 2. All analyses were conducted using SYSTAT 10.2 (Richmond, CA). All P-values are for 2-sided tests.

Results

The optic cup volumes, maximum optic cup depths, and cup/disk area ratios were each significantly smaller

Table 2 ONH indices: between-group comparisons (subjects 40–69 years old)

	ANOVA (Kruskal–Wallis)	Anastrozole versus tamoxifen	Tamoxifen versus control	Anastrozole versus control
Cup volume	***P* = 0.021**	***P* = 0.007**	*P* = 0.052	*P* = 0.560
Maximum cup depth	***P* = 0.016**	***P* = 0.008**	*P* = 0.036	*P* = 0.430
Mean cup depth	***P* = 0.028**	***P* = 0.011**	*P* = 0.050	*P* = 0.645
Cup area	*P* = 0.074	*P* = 0.028	*P* = 0.106	*P* = 0.612
Cup/disk area ratio	***P* = 0.030**	***P* = 0.013**	*P* = 0.045	*P* = 0.763
Cup shape	*P* = 0.593	*P* = 0.325	*P* = 0.797	*P* = 0.429
Rim area	*P* = 0.115	*P* = 0.173	*P* = 0.053	*P* = 0.492
Rim volume	*P* = 0.084	*P* = 0.059	*P* = 0.061	*P* = 0.940
Disk area	*P* = 0.510	*P* = 0.474	*P* = 0.653	*P* = 0.232
Mean RNFL thickness	*P* = 0.718	*P* = 0.654	*P* = 0.438	*P* = 0.735
RNFL cross sectional area	*P* = 0.562	*P* = 0.573	*P* = 0.287	*P* = 0.721
Height variation contour	*P* = 0.617	*P* = 0.375	*P* = 0.893	*P* = 0.387

Left-most data column—*P*-values for the non-parametric ANOVAS (Kruskal–Wallis) across all three amenorrheic subject-groups simultaneously. Comparisons based on the results for the larger contour circles (see Methods). Right 3 columns—unadjusted *P*-values for the post hoc comparisons (Mann–Whitney *U* tests) between pairs of subject-groups. The top 6 rows of variables are for cup data, the next 2 rows are for rim data, and the bottom 4 rows are for disk-margin data. The bold entries signify *P*-values that are considered statistically significant. For the ANOVAS, statistical significance was considered to be $p \leq 0.05$. For the post-hoc comparisons, a step-down Bonferroni approach was used, so that the first (i.e., the most significant) comparison required a significant ANOVA plus $P \leq 0.05/3$, the second most significant comparison required a significant first comparison plus $P \leq 0.05/2$, and the third most significant comparison required a significant second comparison plus $P \leq 0.05$. ''RNFL'' signifies retinal nerve fiber layer thickness. The height variation contour signifies the maximal minus the minimal retinal nerve fiber layer thickness, as defined in the text

for the tamoxifen users than for the anastrozole users. In contrast, none of the comparisons between anastrozole users and control subjects approached significance for any cup dimension. Table 2 provides *P*-values for the between-group comparisons for every summary ONH index. The cup-volume results are represented graphically as a function of age in Fig. 1, which presents the data from the three groups of amenorrheic subjects: tamoxifen users (filled symbols), anastrozole users, (shaded symbols), and control subjects (unfilled symbols).

The data in Fig. 1 indicate that among tamoxifen users, cup volumes tended to be relatively small mainly for those subjects older than about 50 years. Indeed, cup volumes decreased significantly with age (Spearman $r = -0.41$, $P = 0.018$) among tamoxifen users. However, a correlational analysis would necessarily reduce or obscure the significance of any age-related change if such change occurred predominantly within a narrow age range. Thus, although the corresponding results for cup area (Spearman $r = -0.33$), maximum cup depth (Spearman $r = -0.30$), and cup/disk area ratio (Spearman $r = -0.34$) were each non-significant when assessed using correlations, the cup volumes, cup areas, maximum cup depths and cup/disk area ratios were each significantly lower ($P < .05$) for subjects older than 50 years compared to subjects 50 years and younger when based on between-group comparisons of central tendencies. An age cutoff at 51 rather than 50 years yielded the same result. For each of these 2 age cutoffs, the difference in cup volumes between the older and younger tamoxifen users was highly significant ($p \leq .005$).

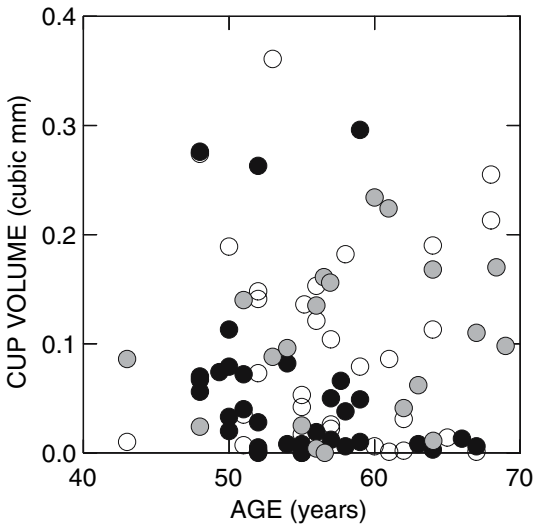

Fig. 1 Graph of cup volume versus age for all amenorrheic subjects. Filled circles represent tamoxifen users, shaded circles represent anastrozole users, and unfilled circles represent control subjects

Among control subjects and anastrozole users, there were not enough younger subjects to make meaningful comparisons between these same 2 age classes. However, we also tested an additional group of women without breast cancer histories, comprised of subjects who met all the same eligibility criteria as the control group, except for the absence of menses. None of the ONH indices differed significantly between the amenorrheic control group (32 women with an average age of 57.2 years) and this additional group (19 women with an average age of 48.0 years), and there was no suggestion of any differences in the cup indices specifically. For example, the median cup volume equaled 0.077 mm^3 for the 32 control subjects and 0.076 mm^3 for the 19 subjects still experiencing menses. Similarly, the median cup/disk area ratio equaled 0.20 for the 32 control subjects and 0.21 for these 19 additional subjects. Among the 32 control subjects themselves, cup volume was not correlated with age (Spearman $r = -0.08$). Among anastrozole users, Spearman $r = 0.27$ ($P = 0.255$), but the regression line relating cup volume to age accounted for only 5% of the total variance, and for only 2% when the calculation was restricted to subjects older than 50 years.

Because cup dimensions varied appreciably with age only for tamoxifen users, and because this variation appeared to be quite marked at about age 50 years, we recomputed the between-group comparisons of all the ONH indices for subjects older than 50 years. (None of the 19 women still experiencing menses were included in any of these comparisons). The results are shown in Table 3. As expected, all of the between-group differences that were significant when the data were compared for all subjects remained significant when the data were compared for only the older subjects, and many of the comparisons between tamoxifen users and control subjects became significant (e.g., for cup volume). In addition, rim volumes (i.e., the volume above and outside the cup but within the disk) now differed significantly between groups when compared for all three groups simultaneously, but not when compared for any two groups on subsequent post hoc analysis. Had we used a less conservative means of comparison (a parametric ANOVA followed by Tukey's HSD test), the rim volumes of the tamoxifen users would have been considered to be significantly larger than the rim volumes of the anastrozole users and also than the rim volumes of the control users. This is probably the appropriate interpretation, since rim volumes, unlike cup volumes, were fairly normally distributed, allowing the use of parametric statistics.

Table 3 ONH indices: between-group comparisons (subjects 51–69 years old)

	ANOVA (Kruskal–Wallis)	Anastrozole versus tamoxifen	Tamoxifen versus control	Anastrozole versus control
Cup volume	***P* = 0.006**	***P* = 0.003**	***P* = 0.019**	$P = 0.330$
Maximum cup depth	***P* = 0.007**	***P* = 0.005**	***P* = 0.014**	$P = 0.309$
Mean cup depth	***P* = 0.016**	***P* = 0.007**	$P = 0.038$	$P = 0.463$
Cup area	***P* = 0.025**	***P* = 0.009**	$P = 0.051$	$P = 0.431$
Cup/disk area ratio	***P* = 0.011**	***P* = 0.005**	***P* = 0.020**	$P = 0.540$
Cup shape	$P = 0.340$	$P = 0.146$	$P = 0.400$	$P = 0.463$
Rim area	$P = 0.113$	$P = 0.161$	$P = 0.049$	$P = 0.511$
Rim volume	***P* = 0.030**	$P = 0.018$	$P = 0.035$	$P = 0.678$
Disk area	$P = 0.435$	$P = 0.301$	$P = 0.883$	$P = 0.217$
Mean RNFL thickness	$P = 0.542$	$P = 0.350$	$P = 0.336$	$P = 1.000$
RNFL cross sectional area	$P = 0.482$	$P = 0.369$	$P = 0.259$	$P = 0.878$
Height variation contour	$P = 0.341$	$P = 0.160$	$P = 0.775$	$P = 0.242$

Same as Table 2, except based on data from subjects older than 50 years)

Table 4 ONH indices: means and standard errors of the mean, and medians (subjects 51–69 years old)

	Anastrozole $n = 18$	Tamoxifen $n = 25$	Control $n = 29$
Cup volume (mm^3)	0.107 ± 0.017 0.104	0.043 ± 0.015 0.012	0.090 ± 0.017 0.073
Maximum cup depth (mm)	0.611 ± 0.047 0.591	0.423 ± 0.040 0.378	0.559 ± 0.040 0.594
Mean cup depth (mm)	0.207 ± 0.017 0.206	0.145 ± 0.014 0.130	0.189 ± 0.014 0.198
Cup area (mm^2)	0.476 ± 0.059 0.523	0.275 ± 0.050 0.139	0.423 ± 0.057 0.385
Cup/disk area ratio	0.207 ± 0.022 0.229	0.123 ± 0.021 0.079	0.193 ± 0.022 0.192
Cup shape	–0.229 ± 0.015 –0.229	–0.199 ± 0.012 –0.205	–0.219 ± 0.013 –0.210
Rim area (mm^2)	1.724 ± 0.066 1.797	1.895 ± 0.071 1.867	1.697 ± 0.061 1.732
Rim volume (mm^3)	0.453 ± 0.025 0.461	0.593 ± 0.042 0.580	0.475 ± 0.028 0.506
Disk area (mm^2)	2.200 ± 0.094 2.316	2.169 ± 0.074 2.156	2.120 ± 0.067 2.154
Mean RNFL thickness (mm)	0.245 ± 0.010 0.246	0.264 ± 0.014 0.262	0.246 ± 0.008 0.242
RNFL cross sectional area (mm^2)	1.281 ± 0.059 1.282	1.366 ± 0.014 1.335	1.266 ± 0.049 0.242
Height variation contour (mm)	0.359 ± 0.019 0.375	0.401 ± 0.019 0.388	0.383 ± 0.015 0.391

Table 4 presents the means and standard errors of the mean, along with the corresponding medians, for every ONH index for each of the three groups of subjects restricted to women older than 50 years. Note that the means and medians for many ONH indices (e.g., rim volume) were similar within groups, but that for some ONH indices (e.g., cup volume), means and medians were dissimilar, signifying a skewed distribution. Thus, Figs. 2–6 present between-group comparisons of medians with interquartile intervals plus overall ranges for cup volume (Fig. 2), cup area (Fig. 3), maximum cup depth (Fig. 4), cup/disk area ratio (Fig. 5), and rim volume (Fig. 6), in all cases for subjects older than 50 years. Although the cup areas of the tamoxifen users were not significantly smaller than those of the control subjects, their cup/disk ratios were significantly lower, probably because a major source of anatomic variability unrelated to tamoxifen use now was factored out.

When the between-group comparisons of the ONH indices were further restricted by excluding tamoxifen and anastrozole users using these medications for less than 1 year, every comparison that was significant (bold numerical entries in Table 3) remained significant. In addition, the duration of medication use was observed to matter little or not at all for the

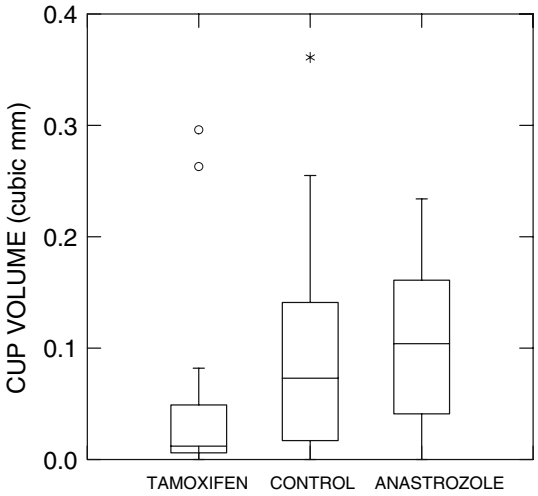

Fig. 2 Box plot of cup volume for women older than 50 years for each of the three amenorrheic subject groups. For each group, the horizontal line inside the box represents that group's median value. The bottom and top of the box represent the upper and lower hinges, respectively, i.e., the first and third quartiles between which lie 50% of all the values. The whiskers represent the range of values that fall within a distance D from the hinges, where D equals 1.5 times the inter-quartile range. Asterisks represent individual points that fall between a distance of D and $2D$ from the hinges. Open circles represent individual points that are more distant outliers

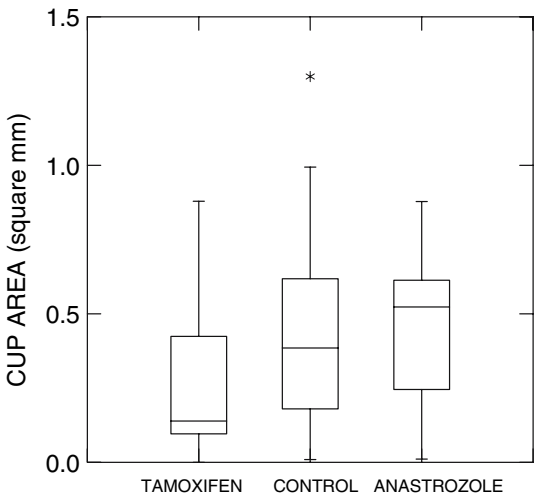

Fig. 3 Same as Fig. 2, except that box plots are for cup areas

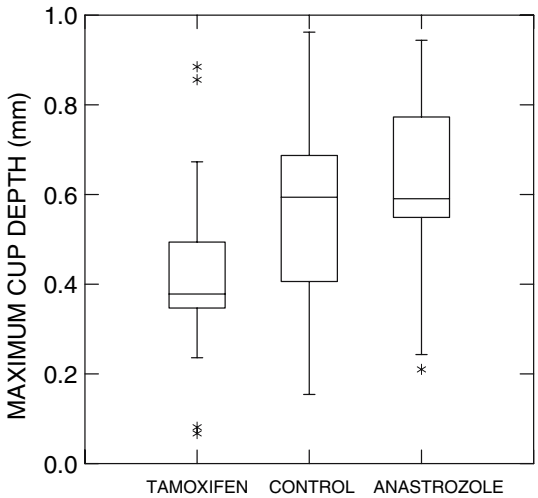

Fig. 4 Same as Fig. 2, except that box plots are for maximum cup depths

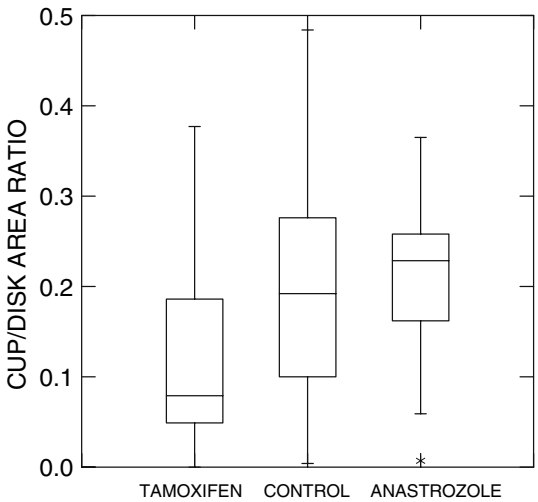

Fig. 5 Same as Fig. 2, except that box plots are for cup/disk area ratios

anastrozole users. For instance, the regression line relating cup volume to the duration of anastrozole use accounted for only 5% of the total cup-volume variance among all 20 anastrozole users, and for only 4% when the calculation was restricted to the 18 anastrozole users older than 50 years. These several sets of results indicate that the cup differences between the tamoxifen and anastrozole groups did not occur because the anastrozole users had experienced too short a period of medication use.

The bottom 4 indices listed in Tables 2–4 represent biomorphometric assessments at the disk margin alone. These are: (1) disk area, (2) mean retinal nerve fiber layer (RNFL) thickness, (3) RNFL cross-sectional area, and (4) the height variation contour, calculated as the maximal minus the minimal RNFL thickness along the contour line. None of these indices differed significantly between groups, although there was some suggestion of greater RNFL thickness for the tamoxifen users (see Table 4).

As stated in the Methods section, we also conducted analyses using the ONH indices derived from contour lines placed slightly interior to the anatomic disk

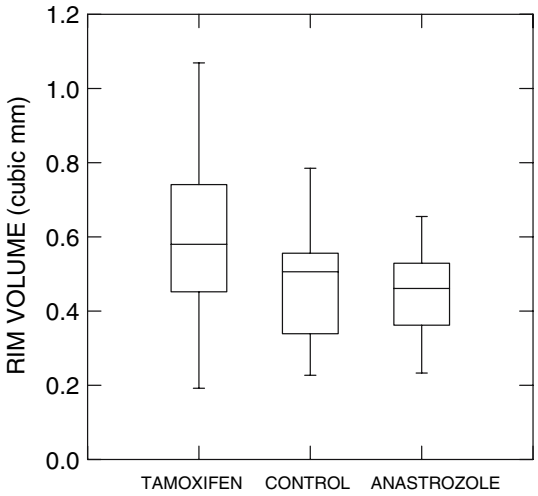

Fig. 6 Same as Fig. 2, except that box plots are for rim volumes

margin rather than exactly at the disk margin. Every statistically significant result concerning the cup remained significant when based on the smaller contours. Conversely, whereas some of the cup-area comparisons involving tamoxifen users were not statistically significant when based on the larger contours (see Tables 2, 3), every corresponding comparison became significant when based on the smaller contours. In particular, the cup-area values for the older tamoxifen users (>50 years) became significantly lower than the cup-area values for the older control subjects (>50 years), presumably because using the smaller contours enhanced the importance of effects occurring predominantly within the interior of the cup.

Discussion

Because tamoxifen users and anastrozole users share similar medical histories, the results indicate that tamoxifen often leads to reductions of optic-cup size among middle-aged women older than about 50 years. Any effects of anastrozole at the ONH remain unproven, and if they exist at all, they are probably minor and opposite to the effects of tamoxifen. In contrast to tamoxifen [9, 10], there seem to be no reports in the peer-reviewed literature concerning any visual or ocular side effects of anastrozole. One study has found that anastrozole can cause regression of breast cancer metastases within the choroid [31].

Because all of the women in this study met a rigorous set of criteria for excellent ocular health, any cup-size changes presumably would be designated as subclinical. Data from several reports suggest that the color vision of tamoxifen users is often altered [10, 19, 20], although such alterations either are subtle or can be revealed only under specialized laboratory conditions, and there is no evidence that color vision changes are caused by effects occurring at the ONH. A recent survey found that about 13% of tamoxifen users reported experiencing vision changes that they attributed to tamoxifen [32], but the nature of these purported changes was not specified, nor were the subjects' reports related to the results of any eye examination. Nevertheless, the subjects reporting vision changes had significantly higher serum levels of tamoxifen and a potent tamoxifen metabolite (N-DMT) than did the women not reporting these changes [32]. In contrast, reports of well-known side effects associated with tamoxifen use, such as hot flashes and vaginal dryness, were not related significantly to these same serum levels [32]. Cases of tamoxifen-induced optic neuropathy have been described in the literature [12, 33–38], but such cases usually are considered rare [9, 10, 38]. Perhaps tamoxifen users sometimes experience a degree of optic neuropathy that would not be detected or confirmed on a presenting eye examination owing to the wide range of normal cup appearances. If so, the results of this study point to a potential new application for intraocular imaging devices such as the HRT, which provides spatial resolution at the ONH on the order of tens of microns and is designed to detect longitudinal change [39]. Several types of high-resolution intraocular imaging device are now available clinically [39], and they allow high-resolution biomorphometric data to be collected in ways that are non-invasive and not intimidating to patients. The ease of data collection with these devices may allow patients who report tamoxifen-related vision change to be evaluated before and after cessation of tamoxifen use, and also after any rechallenge. Until this is done, and then only if changes in vision are shown to correspond to anatomical changes, might it be justified for the type of results described in this study to direct therapy. Presently, the only assured clinical application is to alert ophthalmologists and neurologists that assessment of the ONH for conditions such as glaucoma or optic neuritis may be complicated by the use of tamoxifen.

A human-subjects study such as ours cannot definitively identify the mechanism(s) by which tamoxifen may reduce optic-cup size in vivo. Nevertheless, there is evidence to suggest that tamoxifen might reduce cup size via astrocytic swelling. First, tamoxifen is a potent blocker of swelling- (or "volume-") activated chloride channels for astrocytes assessed in culture [16, 40], and astrocytes are abundant in the optic cup [18, 41]. Second, although astrocytes are present in the RNFL [17,

41], they comprise proportionally more of the neural volume as the cup is descended [41], and astrocytes along with ganglion cell axons are abundant at the base of the cup and also within the sides [42]. However, if the ganglion cell axons increased in volume, one would expect more visual dysfunction than is observed. In addition, there is evidence that tamoxifen-induced development of posterior subcapsular cataracts [10] may involve the blockage of volume-activated chloride channels [43], and that the utility of high-dose tamoxifen as salvage therapy for astrocytomas [44] may depend partly on the ability of tamoxifen to cause astrocytic swelling [45]. Based on photographic assessment of the ONH and retina by a neuro-ophthalmologist (author JF), there did not appear to be any difference in the vasculature of the three subject groups that would have led to the between-group cup differences quantified in this study and also observed in the ONH photographs when examined for groups of subjects rather than for isolated subjects.

The presence of a steep reduction of cup size with age at about 50–51 years among the tamoxifen users suggests that any ability of tamoxifen to reduce cup size, by whatever means, depends on the absence or near absence of natural female hormones, given that the median age of natural menopause is 51 years [25] and given that tamoxifen elevates the estrogen levels of pre-menopausal women [46]. Hormone replacement therapy might, in principle, prevent or alleviate tamoxifen-induced reductions in cup size, but hormone replacement is generally contraindicated for breast cancer survivors, especially for women who have had hormone-receptor-positive tumors [47] (although diverse opinions exist [48]). Hormonal reduction by itself, whether occurring naturally or induced by anastrozole, does not appear to result in cup-size reductions of nearly the magnitude or the frequency inferred for the older tamoxifen users, at least not for the age range that we investigated. The presence of small cups among the tamoxifen users probably did not result from selectively low IOP levels, since the measured IOPs for each subject group were quite similar, differing by no more than 0.2 mmHg on average. Because the presence of small cups among tamoxifen users appeared to be unaccompanied by corresponding significant effects at the disk margin, the effects of tamoxifen on the ONH may be regarded as predominantly localized to the cup.

For this study, we found no evidence of any age-related change of ONH indices for healthy women not using hormonally acting medications. However, a recent study of hundreds of subjects has documented the presence of ONH differences between healthy men and women older than 65 years [49]. In that study, several ONH indices were found to differ significantly between men and women, but the greatest percentage difference was for cup area, which averaged about 14% smaller for women than for men [49]. There is separate evidence that cup areas can depend slightly on the phase of the natural menstrual cycle [50]. These 2 sets of results along with our own results suggest that further studies be conducted to elucidate the effects of hormonally acting medications on the ONH. Such studies should include elderly women using tamoxifen, if possible, since elderly women have higher circulating levels of tamoxifen than do younger women [51].

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