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Review

REVIEW

The Effect of Cancer Treatments on Telomere Length: A Systematic Review of the Literature

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REVIEW

Abstract

Background: It has been hypothesized that cancer treatments cause accelerated aging through a mechanism involving the shortening of telomeres. However, the effect of cancer treatments on telomere length is unclear.
Methods: We systematically reviewed the epidemiological evidence evaluating the associations between cancer treatment and changes in telomere length. Searches were performed in PubMed for the period of January 1966 through November 2016 using the following search strategy: telomere AND (cancer OR tumor OR carcinoma OR neoplasm) AND (survivor OR patient). Data were extracted and the quality of studies was assessed.
Results: A total of 25 studies were included in this review. Ten were solid cancer studies, 11 were hematological malignancy studies, and 4 included a mixed sample of both solid and hematological cancers. Three of the 10 solid tumor studies reported a statistically significant association between cancer treatment and telomere length shortening, and one reported longer telomere length after treatment. Among the hematological cancer studies, three showed statistically significant decreases in telomere length with treatment, and two showed elongation. When these studies were rated using quality criteria, most of the studies were judged to be of moderate quality.
Conclusions: The findings from this review indicate that the effect of cancer treatment on telomere length may differ by cancer type and treatment as well as other factors. Definitive conclusions cannot be made based on the published literature, because sample sizes tended to be small; treatments, cancer types, and biospecimens were heterogenous; and the length of follow-up times differed greatly.

Telomeres are a series of noncoding repeated DNA sequences at the ends of chromosomes that are essential to chromosome stability and integrity (1). Telomeres act as a "mitotic clock" determining the replicative capacity of a cell; when the telomere length of a cell reaches a critically short length, the cell becomes senescent. Telomeres decrease in length with each somatic cell division, and, thus, progressively shorten with age (2,3). Shorter leukocyte telomere length (LTL) has been shown to be associated with earlier mortality (4) and age-related chronic diseases such as cardiovascular disease (5), diabetes (6), and decreased cognitive function (7). Therefore, LTL has been proposed to be a biomarker of aging and is increasingly used in epidemiological studies of age-related diseases.

Both genetic and environmental factors appear to play a role in an individuals' age-related telomere attrition. For example, cigarette smoking, air pollution, and other chronic stressors have been shown to be associated with shorter LTL or accelerated LTL shortening in the general population (8–10). It has also been hypothesized that chemotherapeutic agents with genotoxic effects adversely affect telomeres in cancer patients and survivors (11); however, findings in the literature, overall, regarding the association between cancer treatment and changes in telomere length have been inconsistent, with some studies reporting shortening of telomeres after treatment (12–14) and others elongation (15) or no association (16,17).

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The purpose of this report was to systematically review the epidemiological evidence on the effect of traditional cancer treatments on telomere length.

Methods

Study Search

For this report, we sought all evidence on the associations between traditional cancer treatments (not complementary alternative medicine) and telomere length change reported in epidemiological studies. PubMed was searched for the period of 1966 through November 2016 using the following search strategy: telomere AND (cancer OR tumor OR carcinoma OR neoplasm) AND (survivor OR patient). The search was restricted to human studies and articles written in English. In addition, the study team hand-searched the references cited in the articles chosen for data abstraction as well as relevant review articles identified in the search.

Study Selection

The following exclusion criteria were applied to the abstracts identified in the literature search: 1) no original data (reviews, editorials); 2) studies examining telomere length as a cancer risk or prognostic factor only; 3) telomere length correlated with noncancer treatment factors only; 4) complementary alternative medicine therapy examined in relation to telomere length; 5) telomere length not measured; 6) studies not in cancer survivors or patients; 7) studies not in humans; and 8) case reports. The full-text articles of all references selected after applying these criteria were reviewed using the same exclusion criteria, and the eligibility of each abstract and full-text article was assessed independently in a standardized manner by two reviewers. If separate reports from the same study were published, the report with the most updated data was selected for inclusion.

After full-text review, the study team decided that those articles describing a study investigating the effect of hematopoietic stem cell transplant (HSCT) on telomere length, but not any other type of cancer treatment, would also be excluded, because the mechanism for telomere attrition associated with HSCT is possibly linked to cellular replication stress to achieve engraftment and not necessarily to the toxicity of treatment (18). Further, a number of recent reviews have been published examining the effects of HSCT on telomere length (19,20).

Data Abstraction and Quality Assessment

Data abstraction for the selected articles was performed serially by two reviewers using an electronic abstraction database. Data abstracted included study population, study design, treatment examined, telomere length measurement method, and study results. To assess study quality, criteria published in Longnecker et al. (21) for observational studies were adapted. These criteria included whether the study population was clearly specified and defined, whether a nontreated comparison group was included in the study (if applicable), whether the sample size was more than 50 participants, whether there was a longitudinal component to the study, whether potential key confounding variables (such as age) were measured and adjusted for statistically, whether telomere length was measured at two time points for a least some of the subjects, whether telomere length was measured prior to treatment, and whether quality control information was provided for the telomere length assay used. Each item was coded as "1 = Yes," "0.5 = To some extent," and "0 = No/ Unclear/Information not given" according to the information available in the publications. Scores for all quality criteria were added together for an overall quality score; an overall score of 7 or 8 indicated high quality, an overall score of 4 to 6 indicated moderate quality, and an overall score of less than 4 indicated poor quality. Quality criteria scoring disagreements between reviewers were resolved by consensus.

Results

Study Selection

The search yielded 1217 references, of which 1160 were excluded after abstract review. Of the 57 articles obtained for full-text review, 34 studies examined the association between traditional cancer treatments and telomere length. One study was excluded that reported data included in another publication (22), and 11 studies were excluded that investigated the effect of HSCT on telomere length (20,23–32). After full-text review, an additional three eligible references were added based on review of the reference lists (16,33,34). This left 25 studies that met the inclusion criteria (Figure 1) .

Study Characteristics and Findings

Solid Tumor Only Studies.

Ten of the 25 studies included in this review examined the association between a traditional cancer treatment and telomere length among patients diagnosed with a solid tumor (Table 1). Two of these studies were judged to be of overall high quality (14,33); the other 8 were judged to be of moderate quality.

Of the 10 solid tumor studies, one-half (n = 5) were conducted in a sample of breast cancer patients only (12,16,17,33,35) (Table 1). Only one of these studies showed a statistically significant difference or change (decrease) in telomere length associated with cancer treatment. Specifically, Benitez-Buelga et al. (12), in a cross-sectional analysis, measured LTL of 253 sporadic breast cancer cases (ie, cases that were not suspected to be due to an inherited susceptibility to cancer) at different time points during and post chemotherapy administration [with either a doxorubicin (A), cyclophosphamide (C), and paclitaxel (T) regimen (AC+T/T+AC) or a 5-fluorouracil (F), epirubicin (E), cyclophosphamide (C), and paclitaxel (T) regimen (FEC+T/T+FEC)] and showed a statistically significant negative correlation between number of days on treatment and mean LTL. The correlation was stronger among the breast cancer patients with FEC-based treatment compared with AC-based treatment. However, no LTL measurement was made in pretreatment samples. In a cross-sectional analysis of 236 familial breast cancer cases, Benitez-Buelga et al. (12) also reported that mean LTL was statistically significantly shorter compared with healthy controls during treatment, but not post-treatment (no details on regimen provided). A longitudinal analysis among a subset of seven patients in this study showed elongation of LTL assessed during treatment and again seven years later; the elongation speed was statistically significantly different from the normal shortening of the comparison group. The other four breast cancer studies (16,17,33,35), all of which had a longitudinal component [three with pre- and

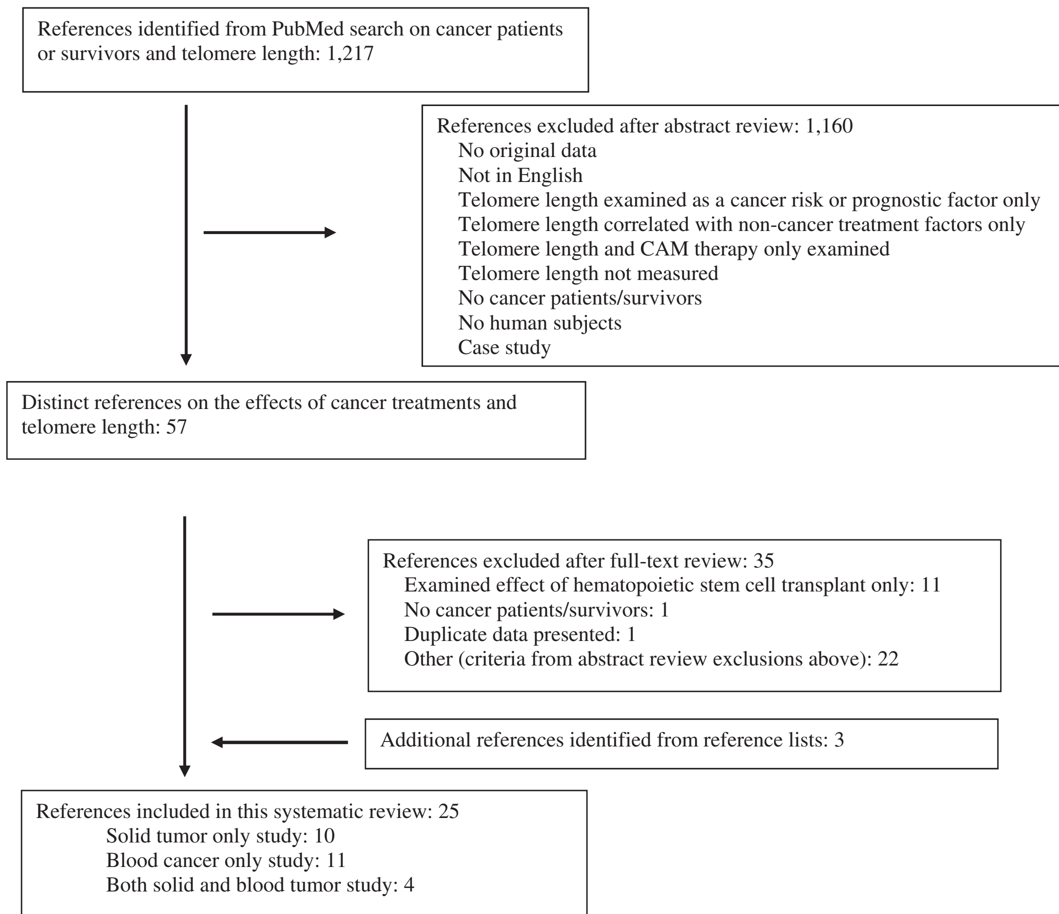

Figure 1. Flowchart of manuscript selection process. CAM = complementary alternative medicine.

posttreatment LTL measurements (17,33,35) and one with LTL measurements 6 to 30 months postdiagnosis (16)], showed no statistically significant association between cancer treatment and LTL.

The other five solid tumor studies were of cancer types other than breast cancer (Table 1). Two were conducted among ovarian cancer patients (38,39); one was conducted among head, neck, and nasopharyngeal cancer patients (14); and the remaining two studies were among patients diagnosed with different types of solid tumors (36,37). Of these five, two studies showed overall statistically significant decreases in telomere length associated with cancer treatment (14,37), one reported longer telomere length after treatment (38), and two reported no overall association between cancer treatment and telomere length, although both these studies reported finding shorter telomere length in a subset of patients (36,39).

Both Yoon et al. (37) and Unryn et al. (14) showed decreases in telomere length associated with cancer treatment. In a sample of 32 patients diagnosed with different types of solid tumors (gastric, esophageal, hepatoma, lung, breast, colorectal, or ovarian), Yoon et al. (37) reported that telomere length measured in peripheral blood mononuclear cells (PBMCs) was statistically significantly shorter after four and six cycles of chemotherapy compared with pretreatment. Further, when compared with age-matched healthy controls, mean PBMC telomere length was found to be statistically significantly shorter at all time points (prechemotherapy, after two, four, and six cycles of treatment) for the cancer patients. Similarly, Unryn et al. (14) investigated PBMC telomere length in a sample of 20 head and neck or nasopharyngeal patients and reported a statistically significant decrease in mean telomere length over time, assessing telomere length before, at day 29, and after the completion of radiation and cisplatin chemotherapy. The mean change in PBMC telomere length was greater among older (> 55 years of age) compared with younger patients.

The two ovarian cancer studies showed variable results, although telomere length was measured in different specimen types in these studies (Table 1). Idei et al. (38) reported elongation of telomere length, measured in cell-free plasma DNA as an indicator of tumor burden, after completion of treatment with cisplatin-based multidrug chemotherapy compared to presurgery among late-stage ovarian cancer patients. The authors hypothesized that the elongation of telomere length posttreatment is because shorter tumor telomere restriction fragments are cleared from circulation during treatment and recuperation (38). Takahashi et al. (39) reported no change in telomere length, measured in ovarian cancer tissue, among responders to a chemotherapy regimen of cisplatin, doxorubicin, and cyclophosphamide, but noted decreases in telomere length among nonresponders. No pre- and posttreatment analyses were presented in Takahashi et al. (39) for the entire sample.

Similar to Takahashi et al., Maeda et al. (36) found no overall statistically significant change in mean LTL associated with radiation treatment among 25 patients with lung, thyroid, prostate, rectal, or hepatoma cancer and no difference in the magnitude of LTL change when comparing cancer cases

Table 1. Solid tumor studies in adults examining the effect of cancer treatment on telomere length (N = 10)

Reference	Cancer type(s)	Study design	Cancer patient sample size	Age range, y	Treatment type	Specimen	Telomere length method	Overall association
Brouwers et al., 2017 (33)	Breast	Longitudinal	109	70–90	Chemo: docetaxel+C; endocrine therapy, radiation, G-CSF	PB leukocytes	qPCR	No association
Benitez-Buelga et al., 2015 (12)	Breast	Longitudinal, cross-sectional*	489	20–87	Chemo: AC+T/T+AC or FEC+T/T+FEC (sporadic cancers only)	PB leukocytes	qPCR, high throughput Q-FISH	Shorter telomere length with treatment†
Duggan et al., 2014 (16)	Breast	Longitudinal, cross-sectional*	611	18–64	Varied, included 5-FU, A, C, taxanes; radiation	PB leukocytes	qPCR	No association
Sanoff et al., 2014 (35)	Breast	Longitudinal	33	32–69	Chemo: AC or ACT; FEC (n = 1)	PB leukocytes	TRF analysis	No association
Maeda et al., 2013 (36)	Lung, thyroid, prostate, rectal, hepatoma	Longitudinal	25	52–83	Radiation	PB leukocytes	TRF analysis	No overall association; decrease in proportion of short telomeres with higher dose†
Yoon et al., 2007 (37)	Gastric, esophageal, hepatoma, lung, breast, colorectal, ovarian	Longitudinal	32	31–65	Chemo: varied based on cancer type	PBMC	TRF analysis	Shorter telomere length after treatment†
Unryn et al., 2006 (14)	Head and neck	Longitudinal	20	44–75	Chemo: cisplatin; radiation	PBMC	TRF analysis	Shorter telomere length after treatment†
Idei et al., 2002 (38)	Ovarian	Longitudinal, cross-sectional	42	NR	Surgery, chemo: cisplatin-based	Free plasma DNA	TRF analysis	Longer telomere length after treatment
Schroder et al., 2001 (17)	Breast	Longitudinal	33	29–54	Radiation, chemo: FEC or FEC+C /thiotepa/ carboplatin and autologous PBSCT, G-CSF; tamoxifen	PB leukocytes	TRF analysis	No association
Takahashi et al., 2000 (39)	Ovarian	Longitudinal	21	28–78	Chemo: A, cisplatin, C	Ovarian tumor tissues	TRF analysis	Shorter telomere length after treatment among nonresponders; no association in responders

*No pretreatment sample measured for telomere length. A = doxorubicin; C = cyclophosphamide; FEC = 5-fluorouracil, epirubicin, cyclophosphamide; 5-FU = 5-fluorouracil; G-CSF = granulocyte colony-stimulating factor; PB = peripheral blood; PBMC = peripheral blood mononuclear cells; PBSCT = peripheral blood stem cell transplant; qPCR = quantitative polymerase chain reaction; Q-FISH = quantitative fluorescence in situ hybridization; T = paclitaxel; TRF = terminal restriction fragment.

†Result statistically significant.

receiving radiation compared with a hospital-based comparison group. However, the data showed a statistically significant decrease in the proportion of short telomeres (defined as those <4.4 kb) with increasing daily radiation dose. The authors suggested two possible explanations: only short telomeres are subjected to the telomere-elongating mechanism or short telomeres disappear from the telomere length distribution with radiation therapy (36).

Hematological Malignancy Only Studies

Eleven of the 25 studies included in this review examined the association between a traditional cancer treatment and telomere length among patients diagnosed with a hematological malignancy (Table 2). Ten of the studies were judged to be of overall moderate quality; one was judged to be of poor quality based on the criteria assessed (48). The types of hematological cancers investigated were chronic myeloid leukemia (CML) in four studies (40,44,46,47), non-Hodgkin lymphoma (NHL) in three studies (41,43,45), and acute promyelocytic leukemia (15), Hodgkin lymphoma (42), acute lymphocytic leukemia (ALL) (48), and lymphoma, not otherwise specified (13), in one study each. Ten of the blood cancer-only studies were conducted on adult patients; Nowak et al. (48) was conducted in a sample of childhood cancer patients. Nine studies examined telomere length in peripheral blood cells (eg, leukocytes, mononuclear cells, or progenitor cells) (15,34,40,42–46,48) and two measured telomere length in bone marrow mononuclear cells (41,47).

Among the nine studies examining telomere length in peripheral blood cells, three reported statistically significant shortening of telomere length associated with treatment (13,43,45) (Table 2). In a study of PBMC telomere length change in patients with NHL, Lee et al. (45) showed a statistically significant decline in mean telomere length among five NHL patients with telomere length measurements before and after a CHOP-based (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy regimen, although there was no correlation between change in telomere length and time since the end of chemotherapy. Mean telomere length both before and after chemotherapy was shorter among the NHL patients (five with pretreatment and 15 with posttreatment measurements) compared with 39 age-matched healthy individuals, and telomere length attrition was suggested to be greater among the cancer patients. However, a statistical comparison examining change over time between the two groups was not presented. Szyper-Kravitz et al. (13) reported that among five lymphoma patients treated with CHOP and not administered granulocyte colony stimulating factor (G-CSF), mean telomere length decreased statistically significantly after two cycles of treatment. Conversely, in the same study, among a separate group of five lymphoma patients treated with CHOP and G-CSF, telomere length was preserved or increased. The investigators suggest these findings were possibly due to upregulation of telomerase activity by G-CSF (13). Finally, Ricca et al. (43) measured telomere length in peripheral blood progenitor cells among 37 NHL patients receiving high-dose cyclophosphamide and high-dose cytarabine (Ara-C) prior to autograft and showed statistically significant shortening in mean telomere length after high-dose Ara-C compared with before Ara-C administration (but after receipt of high-dose cyclophosphamide).

Although *P* values or other measures of statistical significance were not reported, Drummond et al. (44) reported that mean LTL among CML patients treated with imatinib was shorter in nonresponders (n = 11) but longer in responders (n = 10), eight months and six months after treatment initiation, respectively. Two additional studies showed statistically significant increases in telomere length associated with treatment. Brummendorf et al. (46) found in 119 CML patients with sequential measurements a statistically significant increase in mean peripheral blood granulocyte telomere length when measured after, compared with before, imatinib treatment. Further, mean telomere length was statistically significantly shorter in 197 CML patients early after treatment initiation (<144 days) than in 196 patients with longer treatment duration (>144 days), indicating possible elongation of telomere length with longer imatinib treatment duration (46). Ghaffari et al. (15) reported that, in a sample of 40 patients diagnosed with acute promyelocytic leukemia, telomere length tended to increase as patients responded to standard intensive remission induction and courses of consolidation therapy with arsenic trioxide. Of note, the findings of telomere lengthening associated with treatment in the Brummendorf et al. (46), Ghaffari et al. (15), and Drummond et al. (44) (for responders) studies may be explained by a decrease in the number of malignant cells with shorter telomere lengths over time (ie, a decrease in tumor load) rather than a direct effect of treatment; however, this has not been studied.

The remaining three studies examining peripheral blood cell telomere length either showed no statistically significant change in mean telomere length associated with treatment (42) or did not statistically assess or report change (or before/after treatment differences) in telomere length associated with treatment (40,48). One of the two studies that examined telomere length in bone marrow mononuclear cells reported decreases in mean telomere length associated with specific treatment regimens. Guidetti et al. (41) reported that mean telomere length declined in 54 NHL patients treated with high dose-radioimmunotherapy (^{90}Y-ibritumomab tiuxetan after high-dose cyclophosphamide and/or high-dose Ara-C) when measured at three different time points post, compared with pre-, radioimmunotherapy treatment. No values of statistical significance (eg, *P* values) were presented, nor were prechemotherapy samples or data from controls collected. In the second study that used bone marrow mononuclear cells to study changes in telomere length associated with cancer treatment, no data were shown or reported for the overall telomere length change among 16 patients administered alpha-interferon, although five of the patients were reported to have longer telomere lengths post- compared with pretreatment (47).

Mixed Solid Tumor and Hematological Malignancy Studies

Four of the 25 studies included in this review examined the association between a traditional cancer treatment and telomere length in a sample of patients diagnosed with either a solid tumor or a hematological cancer (Table 3). Three of these studies were judged to be of moderate quality, and one was judged to be of poor quality (49).

Three of the four mixed solid tumor and hematological cancer studies showed decreases in telomere length in at least one group of patients in the study sample (34,50,51) (Table 3). Diker-Cohen et al. (34) reported that, in 14 adult patients diagnosed with NHL, CHOP treatment resulted, at 6 to 12 months postchemotherapy, in an average PBMC telomere length that was approximately 35% that of a control group of 40 age-matched volunteers ($P < .05$). At 2 years, telomere length remained shortened to the same degree (35%) of the age-matched control group. Further, in the NHL patients (n = 10)

Table 2. Blood cancer studies examining the effect of cancer treatment on telomere length (N = 11)

Reference	Cancer type(s)	Study design	Cancer patient sample size	Age range, y	Treatment type	Specimen	Telomere length method	Overall association
Adult								
Lobetti-Bodoni et al., 2012 (40)	Chronic myeloid leukemia	Longitudinal, cross-sectional*	81	23–88	Varied; cytosine-arabinoside, interferon-α, imatinib, dasatinib	PB PMN; monocyte-depleted PBMC	TRF analysis	No association
Guidetti et al., 2011 (41)	Non-Hodgkin lymphoma	Longitudinal*	53	26–76	High-dose radioimmunotherapy based on ^{90}Y-ibritumomab tiuxetan and autograft	Bone marrow mononuclear cells	TRF analysis	Shorter telomere length after treatment
Ghaffari et al., 2008 (15)	Acute promyelocytic leukemia	Longitudinal	40	14–50	Chemo: arsenic trioxide	PBMC	TRF analysis	Longer telomere length after treatment§
M'kacher et al., 2007 (42)	Hodgkin lymphoma	Longitudinal	119	28–76	Radiation, chemo (not specified)	PBMC	TRF analysis	No association
Ricca et al., 2005 (43)	Non-Hodgkin lymphoma	Longitudinal	37	18–59	Chemo: high-dose Ara-C (after high dose C and APO); autograft, G-CSF	PB progenitor cells	TRF analysis	Shorter telomere length after treatment§
Drummond et al., 2004 (44)	Chronic myeloid leukemia	Longitudinal	95	NR†	Imatinib	PB leukocytes	Flow-FISH	Shorter telomere length in nonresponders to imatinib; longer telomere length in responders
Szyper-Kravitz et al., 2003 (13)	Lymphoma	Longitudinal	10	45–80	Chemo: CHOP; G-CSF	PBMC	Flow-FISH	Shorter telomere length after CHOP without G-CSF§; no change or longer in patients treated with CHOP and G-CSF
Lee, et al. 2003 (45)	Non-Hodgkin lymphoma	Longitudinal, cross-sectional	15	19–72	Varied, included CHOP, radiation, ifosfamide, etoposide, carboplatin	PBMC	TRF analysis	Shorter telomere length after treatment§
Brummendorf et al., 2003 (46)	Chronic myeloid leukemia	Longitudinal, cross-sectional	206	16–81	Imatinib	PB granulocytes	Flow-FISH	Longer telomere length after treatment§
Iwama et al., 1997 (47)	Chronic myeloid leukemia	Longitudinal	16	NR‡	alpha-interferon	Bone marrow mononuclear cells	TRF analysis	No association (5 of 16 had normalization of telomere length after treatment)
Pediatric								
Nowak et al., 2006 (48)	Acute lymphocytic leukemia	Longitudinal	29	N/A	Chemo (not specified)	PB lymphocytes	TRF analysis	Only range values before treatment and in remission reported

*No pretreatment sample measured for telomere length. APO = doxorubicin, vincristine, prednisone; Ara-C = cytarabine; C = cyclophosphamide; CHOP = cyclophosphamide, doxorubicin, vincristine and prednisone; FISH = fluorescence in situ hybridization; G-CSF = granulocyte colony-stimulating factor; N/A = not available; NR = not reported; PB = peripheral blood; PBMC = peripheral blood mononuclear cells; PMN = polymorphonucleates; TRF = terminal restriction fragment.

†Age range not reported for entire sample; for a subset of the study sample, the age range was 24 to 77 years.

‡For entire sample of 44 patients reported in the manuscript, the age range was 14 to 72 years; telomere length analyses was limited to a subset of 16 patients.

§Result statistically significant.

REVIEW

Table 3. Blood and solid cancer studies examining the effect of cancer treatment on telomere length (N = 4)

Reference	Cancer type(s)	Study design	Cancer patient sample size	Age Range, y	Treatment type	Specimen	Telomere length method	Overall association
Adult								
Diker-Cohen et al., 2013 (34)	Non-Hodgkin lymphoma, colon, chronic lymphocytic leukemia, lymphoma	Longitudinal	42	45–74	Chemo: varied based on cancer type	PBMC	Flow-FISH	Shorter telomere length with treatment†
Kronenwett et al., 1996 (49)	Non-Hodgkin lymphoma, multiple myeloma, breast cancer, rhabdomyosarcoma	Cross-sectional*	54	22–60	Chemo: varied based on cancer type; G-CSF	PB stem cells; mononuclear cells	TRF analysis	No association
Pediatric								
Franco et al., 2003 (50)	Acute lymphocytic leukemia, Hodgkin lymphoma, Ewing's sarcoma, hepatoblastoma, clear cell sarcoma, neuroblastoma, Wilms tumor, brainstem tumor, astrocytoma, severe aplastic anemia	Longitudinal	24	0.7–16	Chemo: varied based on cancer type, radiation, autologous stem cells	PBMC or bone marrow mononuclear cells and granulocytes	TRF analysis	Shorter PB telomere length with treatment, primarily among solid tumor patients and not hematological cancer patients
Engelhardt et al., 1998 (51)	Acute lymphocytic leukemia, acute myeloid leukemia, sarcoma, Wilms tumor, Hodgkin lymphoma, Central nervous system tumors, hepatoblastoma, germ cell	Longitudinal	25	1–15	Chemo: varied based on cancer type	PBMC and bone marrow mononuclear cells and granulocytes	TRF analysis	Shorter telomere length with treatment

*No pretreatment sample measured for telomere length. FISH = fluorescence in situ hybridization; G-CSF = granulocyte colony-stimulating factor; PB = peripheral blood; PBMC = peripheral blood mononuclear cells; TRF = terminal restriction fragment.
†Result statistically significant.

who received more intensive therapy (six cycles plus, after relapse, an additional four cycles of etoposide, cisplatinum, Ara-C and methylprednisolone), more marked telomere shortening was observed 2 months postchemotherapy compared with the standard therapy group and the controls. Average telomere attrition rates over the course of the study were reported as 50 base pairs (bp)/year in the control group, 250 bp/year in the standard chemotherapy group, and 500 bp/year in the intensive chemotherapy group. In the same study, Diker-Cohen et al. (34) reported statistically significant telomere shortening 2 months after either 5-fluorouracil (5-FU) or fludarabine treatment among 10 adult colon cancer patients and 8 adult low-grade lymphoma or chronic lymphocytic leukemia patients, respectively. After 1 year posttreatment, mean telomere length returned to the pretreatment value among the 5-FU group but not the fludarabine group.

Franco et al. (50) examined several subgroups of pediatric cancer patients. Among 10 ALL patients and 9 solid tumor patients treated with various anticancer regimens based on diagnosis, the authors reported pre- to posttreatment shortening in PBMCs and granulocyte telomere length. The magnitude of changes was greater in the solid tumor compared with the ALL patients, although values of statistical significance were not reported. PBMC and granulocyte telomere length losses associated with treatment (based on diagnosis) were also observed among ALL and solid tumor pediatric patients in a study by Engelhardt et al. (51). Additionally, solid tumor patients in this study who received high-dose chemotherapy ($n = 2$) showed a more pronounced decline in telomere length compared with those who received standard-dose chemotherapy ($n = 7$). A test of statistical significance was not conducted.

Discussion

For this systematic literature review, the epidemiological evidence pertaining to the effect of traditional cancer treatments on telomere length in cancer patients was identified and critically evaluated to determine whether such a relationship exists. However, an assessment of consistency, and, thus, causality, cannot be made using the current body of evidence, because the studies are not directly comparable. One criterion of the Bradford-Hill set of nine criteria that allow for the assessment of the epidemiological literature as to whether a causal relationship exists between a presumed cause and an observed effect is *consistency*, or reproducibility, in epidemiological findings across different studies of different patient populations (52). The identified 25 studies did not examine the same treatment regimens over the same period of follow-up time; it is likely that different types of treatments affect telomere length in different ways or may not affect telomere length at all. Further, the studies included in this review collected different specimens for telomere length measurement, adjusted for different (and in some cases a limited set of) potential confounders (eg, age, cigarette smoking), and measured telomere length change over different lengths of time or at different time points on the cancer treatment continuum (eg, pre- to posttreatment or only posttreatment). In addition, different methods were used to assess telomere length across studies, precluding any comparison of telomere length and changes in telomere length between studies and even across different cancer types or treatments. Because of this lack of comparability, a meta-analysis of this literature could not be conducted; thus, results were reported in a descriptive, albeit systematic, manner.

LTL has been hypothesized to be a biomarker of biological aging, with shorter telomeres being indicative of more advanced biological age (53). Numerous studies have shown that various disease states and exposure to chronic stressors, such as cigarette smoking, accelerate LTL shortening (8,9). LTL has also been shown to be a prognostic marker for cancer survival as well as the development of a number of chronic conditions, including cancer and cardiovascular disease (5,54–57). Thus, if known toxic cancer therapies, which cause cycles of cell injury and repair through a variety of different mechanisms to treat cancer, lead to telomere shortening, this may be the biological mechanism by which cancer patients and survivors are at increased risk for accelerated or premature aging.

Indeed, telomere stability has been shown in in vitro studies to be affected by a number of conventional chemotherapies in a manner that depends on the specific drug mechanism of action. For example, both cyclophosphamide, an alkylating agent that is used in regimens to treat breast and other types of cancer, and cisplatin, an alkylating-like agent used to treat testicular, ovarian, and lung cancers in particular, may directly cause telomere damage and shortening by inducing guanine DNA-DNA cross-links (58). Telomeres are specifically susceptible to these cross-links because of their G-rich DNA sequences (58–60). Mitotic inhibitors, such the taxane class of chemotherapy drugs, can cause telomere uncapping, triggering telomere dysfunction and shortening in cancer cells (11). In contrast, G-CSF has been reported to increase LTL through upregulation of telomerase (13,51). Interestingly, four studies included in this review reported G-CSF as part of the treatment regimen and found no association with telomere length change (13,17,33,49). Thus, there is biological plausibility of a telomere shortening effect of certain conventional cancer therapies that may lead to accelerated aging among cancer patients and survivors. It should also be noted that telomere length is also regulated by telomerase activity, and it is possible that cancer treatments have a shortening effect on telomere length through the downregulation of telomerase activity (61). However, there is a paucity of epidemiological evidence on this relationship. A study by Franco et al. (50) included in this review showed no change in telomerase activity associated with cancer treatment among pediatric solid tumor patients and a decrease in the level of telomerase activity among ALL patients associated with induction therapy that continued through maintenance therapy and after the end of treatment.

Although some of the studies included in this review showed telomere shortening associated with cancer treatment, the results of several studies suggest that the shortening may not be permanent and that there may be a recovery period during which telomere length normalizes or lengthens (12,15,38,44,46,47). However, four of these studies were conducted among leukemia patients, and in these studies, pretreatment telomere length measurement is primarily representative of telomere length in cancer cells. In contrast, telomere length measured in specimens during or posttreatment represents telomere length in a mixed population of normal and leukemic cells (15,44,46,47). Of the two solid tumor studies suggesting posttreatment lengthening of telomere length, the results of Benitez-Buelga et al. (12), which observed LTL elongation among a sample of familial breast cancer patients (BRCA1/2 carriers), were based on a small sample size ($n = 7$) with no treatment details and no pretreatment measurement. The findings of Idei et al. (38) were based on cell-free plasma DNA telomere length measurements. The observed elongation of telomere length in Idei et al. (38) may, like the hematological studies discussed

REVIEW

above, reflect a decrease in circulating tumor cells (and DNA) rather than the result of a direct treatment effect on telomere length.

Most of the studies included in this systematic review measured telomere length in leukocytes; this is the most widely used specimen type for telomere length measurement, especially in large population-based or clinical studies due to its accessibility as a DNA source. However, it is possible that the telomere length of the tumor tissues that are most affected by specific cancer treatments (rather than LTL) may best explain the reported findings of cancer treatments and accelerated aging, of which endpoints often include comorbid conditions and functional decline. Leukocytes have a higher replication rate than cells in somatic tissues, suggesting that LTL may decrease at a faster rate than telomere length in other tissues. However, studies comparing LTL with telomere length in other somatic tissues from the same individuals have shown that although absolute differences exist, there are strong correlations (62–64). These findings are not surprising, because telomere length is heritable (65). It should be noted that the relationship between telomere length in leukocytes and diseased tissues (such as solid tumor tissue) is not clear. Furthermore, as discussed above, the studies examining telomere length in leukocytes taken from patients with hematological malignancies may not be an accurate reflection of the true telomere length because the blood samples are a mix of normal and diseased cells, where the proportion would also differ by disease status. Therefore, researchers planning clinical or epidemiological studies must determine the most appropriate biospecimen to collect and telomere length measurement method to use given the type of cancer to be studied and the specific hypothesis to be tested.

REVIEW

An additional challenge of comparing the results across the published studies is that different methods for quantifying telomere length were used, and the utility of the measure depends on the validity and reliability of the techniques. Those used in the studies reviewed here were terminal restriction fragment visualized by Southern blot, quantitative polymerase chain reaction (qPCR), quantitative fluorescence in situ hybridization (qFISH), and flow-FISH. Each of these methods has its strengths and weaknesses; these methods have recently been reviewed by multiple investigators (66–68). In brief, terminal restriction fragment measurement is currently considered the gold standard for telomere length measurement and can be used to measure telomere length in extracted DNA from stored samples, such as those typically available in epidemiological studies. This method provides an actual kilobase size estimate of telomere length, making it feasible to compare results across studies (66). The limitations of terminal restriction fragment measurement are that it often provides an overestimation of true telomere length because of the inclusion of subtelomeric DNA in the measurement, the need for large amounts of DNA, and lower sensitivity to detect very short telomeres (65). To address some of the limitations of terminal restriction fragment measurement, qPCR was developed as a technique to measure telomere length using smaller amounts of DNA in a high throughput manner, making this technique attractive for studies of larger sample sizes. It produces a telomere length result referenced to a standard single copy gene and not an absolute kilobase length estimate; thus, it is difficult to compare telomere length results using this method across studies. Other limitations of the qPCR method are that that reference standards are lacking and there can be variation between and within laboratory batches (66). Both terminal restriction fragment and qPCR telomere length results are limited in that they reflect an average across the population of cells in the sample. In contrast, flow-FISH can more accurately measure LTL, even within specific cell populations; however, viable cells are needed for this method, thus limiting its use in population-based studies (65). It is therefore important when deciding which method to measure telomere length that several factors be evaluated, including the research question, study population, sample size, biospecimen type, timing of analysis, and available resources (66). Further, minimizing technique-related measurement error is imperative in telomere length studies, underscoring the need for rigorous laboratory quality control procedures (65,66).

Advances in cancer treatment have led to better overall survival rates for cancer patients, resulting in a growing number of cancer survivors (69). Despite surviving their cancer, there is increasing evidence that cancer treatments may lead to an accelerated aging phenotype, putting the cancer survivor at risk for premature death from non-cancer aging-related diseases and poor quality of life. A better characterization of the accelerated aging phenotype, the biological mechanisms associated with its occurrence, and which cancer survivors are most at risk is needed. Importantly, LTL may not be the best measure by which accelerated aging may occur with exposure to certain types of cancer treatments; telomere length measured in the tissue most adversely affected by the cancer treatment should be explored as a biomarker of damage related to a specific aging-related outcome. Thus, when planning clinical and epidemiological studies examining cancer treatment and its effect on aging, investigators should determine whether telomere length, measured either in leukocytes or specific tissues, is the mechanism by which cancer treatment leads to accelerated aging and is a valid biomarker to address the research question. If telomere length is used, it is important to select the appropriate epidemiological study design, which includes considering an untreated comparison group, using an adequate sample size, using an accurate telomere length assay, and adjusting for confounders. Further, measuring telomere length longitudinally is crucial; this includes measurement of telomere length prior to and after treatment. The careful design of such studies examining mechanisms of accelerated aging associated with cancer treatments will lead to better quality epidemiological evidence, with the goal of developing interventions to prevent, mitigate, or even reverse deleterious cancer treatment effects.

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