Telomere dysfunction in Peripheral Blood Lymphocytes from Patients with Primary Sclerosing Cholangitis and Inflammatory Bowel Disease

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List of Abbreviations

PSC  Primary sclerosing cholangitis
IBD  Inflammatory bowel disease
UC   Ulcerative colitis
CRC  Colorectal carcinoma
TA   Telomere aggregates
BBF  Breakage bridge fusion
TC   Telomere capture
TL   Telomere length
CD   Crohn's disease
CRP  C-reactive protein
HBI  Harvey-Bradshow index
TERC Telomerase RNA component
CRP  C reactive protein
PBS  Phosphate buffered saline
SNRPN Small nuclear ribonucleoprotein-associated protein N
FISH Fluorescence in situ hybridization
CEP  Chromosome enumeration probes
HCC  Hepatocellular carcinoma

Conflict of interest: None

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Abstract

Background and aims: Primary sclerosing cholangitis and inflammatory bowel disease are two associated, chronic inflammatory, pre-malignant conditions. We hypothesized that patients with these disorders may harbor telomere dysfunction as a marker of chromosomal instability. The aim of our study was to compare parameters of the telomere-telomerase system in these cohorts.

Methods: In this prospective study, peripheral blood was withdrawn from patients with primary sclerosing cholangitis (N=20), inflammatory bowel disease (N=20) and healthy controls (N=20), and lymphocytes were isolated. Telomere length was quantified as a function of the signal intensity and telomere number. Random aneuploidy and telomere capture were determined by fluorescence in situ hybridization technique with specific probes.

Results: Patients with inflammatory bowel disease had higher measures of intestinal disease activity than patients with primary sclerosing cholangitis. Despite this, shorter telomere length and telomere aggregates, especially the fusion of 2-5 telomeres, were observed at significantly higher rate in patients with primary sclerosing cholangitis relative to inflammatory bowel disease or healthy controls. Rates of aneuploidy and telomere capture were higher in the two probes in both diseases compared to controls (p < 0.001).

Conclusion: Dysfunction of telomeres was demonstrated in primary sclerosing cholangitis patients more than inflammatory bowel disease and healthy controls patients, which attests to genetic instability and immunosenescence.

Key words: primary sclerosing cholangitis, telomere length, genetic instability
Background

Primary sclerosing cholangitis (PSC) is a chronic cholestatic disorder that involves both intra- and extra-hepatic bile ducts. A combination of inflammatory and fibrotic changes leads to a typical cholangiographic pattern of multiple, widespread, bile duct strictures and dilatations, as well as histological features of periportal inflammation and a fibro-obliterative process [1]. The pathogenesis of PSC remains poorly understood, but up to 80% have concomitant inflammatory bowel disease (IBD), mostly ulcerative colitis (UC). One of the hallmarks of the disease, a consequence of the continuous inflammation, is the increased tendency to develop hepatobiliary and extra-hepatic malignancies [2]. The risk of colorectal cancer (CRC) and dysplasia is also higher in IBD with PSC than in IBD alone [2-4].

Telomeres are specialized nucleoprotein complexes that are localized at the physical ends of linear, eukaryotic chromosomes. Telomeres protect the chromosome ends from fusion and degradation, and are important for the normal segregation and maintenance of chromosomes, compensating for the DNA loss and consequent chromosomal shortening with each mitotic cell division [5]. To maintain chromosomal integrity, telomeric repeats are added to the chromosome via the telomerase enzyme, a specialized reverse transcriptase. In humans, telomerase is normally active in germ cells, but not in somatic cells, where telomeric DNA shortens progressively with time as a result of an end-replication mechanism [6]. At a critical point in this process, human somatic cells enter a nonreplicative, but viable state, known as senescence, followed by mitotic crisis and cell apoptosis. Upregulation /activation of telomerase may help to stabilize the telomeres, and in this manner, genetically unstable cells that bypass crisis are immortalized by telomere elongation [5, 7]. Almost all cancer cells (85-90%) are characterized by abnormal re-expression of telomerase and drastically shorter telomeres compared to surrounding healthy tissue [5]. Dysfunctional
telomeres may recombine and fuse, initiating random chromosome breakage and the formation of dicentric chromosomes, thereby increasing chromosome instability and the risk of oncogenesis [8].

One marker of chromosomal instability, seen in various malignant and pre-malignant states, is the tendency to form telomere aggregates (TA), in contrast to the non-overlapping nature of telomeres in normal nuclei. This can be apparent when a three-dimensional (3D) imaging approach is applied, and it signifies not just a transient aberration in the 3D organization of the nucleus, but a true end-to-end fusion [9-10]. Telomere aggregates are formed during a breakage-bridge-fusion (BBF) cycle that contributes to deletions, gene amplification and overall genetic changes that are associated with tumorigenesis [9, 11-12].

In addition to telomerase-mediated chromosome healing, chromosomal integrity can be also maintained by another mechanism, which is telomere capture (TC). Telomere capture, first described by Meltzer et al. in cancer cells [13], is a process by which broken chromosomes can acquire new telomeres from normal chromosomes in order to stabilize by nonreciprocal translocation. Tumors especially are reported to use these two mechanisms to overcome replication-induced telomere shortening [14]. Both TA and TC were demonstrated by our group to be significantly increased in different stages of non-Hodgkin lymphoma and hepatitis C, another pre-malignant condition [15-16].

Aneuploidy is another marker of tumorigenesis. This refers to a chromosomal constitution of cells that deviates from the normal number of chromosomes or chromosome segments, generated by gain or loss of specific chromosomes or genes. According to the aneuploidy-cancer theory, carcinogenesis is initiated by a random aneuploidy, which causes chromosomal destabilization due to imbalance of highly conserved proteins that segregate,
synthesize and repair chromosomes [17-18]. Thus, it is predicted that the chromosomal and genetic instability is proportional to the degree of aneuploidy [14, 18].

Telomere shortening was also demonstrated in metaplasia and dysplasia of biliary duct and in cholangiocarcinoma [19]. Since leukocyte telomere length has been demonstrated to be highly correlating with that in cells from other tissues [20], we hypothesized that patients with PSC display telomere dysfunction in peripheral blood lymphocytes as surrogate marker of genetic instability. Indeed, we have recently reported an amplified telomerase RNA component (TERC), an RNA gene that is a component of telomerase, in lymphocytes of patients with PSC and IBD [21]. In addition, PSC is an autoimmune disease with a potential of telomere-telomerase dysfunction of peripheral immune blood cells. Thus, the aim of the current study was to assess telomere length, aneuploidy and other parameters of telomere dysfunction in these diseases and to evaluate the clinical correlates of this dysfunction.

Patients and methods

Study design
This prospective study was conducted at Meir Medical Center, which is a tertiary referral center for patients with IBD and PSC in Israel. All patients were followed in our outpatient clinic. Exclusion criteria included age less than 18 years, inability to provide an informed consent, pregnancy, known present or personal history of malignancy, previous colectomy, cirrhosis, chronic infectious or autoimmune liver conditions other than PSC, other chronic disease or dysfunction of heart, lungs or kidneys and known diabetes. An age-matched, healthy population, recruited from a general GI clinic, was evaluated as a control group. The diagnosis of IBD was based on the combination of compatible clinical picture and characteristic endoscopic and histopathologic findings, and only patients with colonic involvement were included. The diagnosis of PSC was established by a combination of
clinical picture with typical biochemical and cholangiographic patterns. Liver biopsy (not mandatory for diagnosis) was available for 4/20 patients with PSC, showing characteristic findings. Cirrhosis and portal hypertension were excluded in the rest of PSC patients by cross-sectional imaging and upper endoscopy to eliminate esophageal varices.

For each patient, demographic data, drugs, glycated hemoglobin (Hb A1C), body-mass index (BMI) and smoking habit were collected and colitis activity was assessed by serum C-reactive protein (CRP) and a symptom-based, well-validated questionnaire, either the partial Mayo score for UC [20] or the Harvey-Bradshaw index (HBI) for Crohn's disease [23]. For the partial Mayo score, a score of 0-3 was considered mild disease (grade 1) and higher scores (4-9) were considered moderate-to-severe disease (grade 2-3). An HBI score 0-3 was considered disease remission, score 3-6 mild disease, and score > 6 moderate-to-severe disease. For PSC patients, we used the revised Mayo Risk Model, a well-validated prognostic model that has been used to estimate survival [24], in order to categorize the patients. The risk score was calculated according to 5 variables: age, bilirubin, albumin, aspartate aminotransferase and history of variceal bleeding. Additionally, a blood sample was collected from all subjects for the purpose of telomere analysis, performed from peripheral blood lymphocytes.

The study was approved by the Institutional Ethics Review Board and all participants signed an informed consent form.

Lymphocyte culture

Phytohemagglutinin, 0.2 ml heparin (1000 IU) and 1% antibiotics were added to RPMI 1640 culture medium. After incubation, colchicine (final concentration 0.1 µg/mL) was added to the cultures for 1 hour, followed by hypotonic treatment (0.075 mol/L KC1 at 37°C for 15 minutes). The lymphocyte suspensions of the three samples were stored at -4°C.
Fluorescence in situ hybridization (FISH) technique

As previously described [16, 19], fresh slide spreads were incubated for 10 minutes in 2x standard saline citrate (2x SSC) at 37°C, followed with fixation in formamide (diluted 1:40 in phosphate buffered saline (PBS) and 0.18gr MgCl₂) for 15 min. The slides were washed in PBS for 5 min and incubated in pepsin solution (75 g lyophilized pepsin dissolved in 50 ml HCl 0.01N) followed by a wash in PBS for 5 min.

Telomere length

Telomere length was quantified, as previously described [25], as a function of the signal intensity and telomere number. The cells were first categorized as having high (strong) or low (weak) fluorescence, which is correlated with long and short telomeres respectively, and each group was further categorized by number of telomere signals: 0-10, 11-30, or >30. This yielded a total of six categories, as shown in figure 3. The analysis was done for each study group and the findings were compared.

Telomere capture (TC)

We used a Cytocell for 15qter (SNRPN, red fluorophore, with 15qter control probe, green fluorophore, cat no: LPU005; UK) and a Cytocell probe for 13qter (13q14.3 red fluorophore, with 13qter control probe, green fluorophore, cat no: LPU006; UK). The analysis was performed primarily on interphase nuclei, because most are in interphase. In the nuclei, the numbers of the specific loci of the specific chromosome (SNRPN or 13q14.3, orange, "the normal disomic loci") and its sub-telomeric region (15qter or 13qter, green) were compared. The number of signals of the SNRPN or 13q14.3 locus was compared to the numbers of signals of the sub-telomeric region of the specific chromosome. For example, the normal appearance is two orange and two green signals
(2R:2G), while an abnormal appearance, which represents telomere capture, is two orange compared with one (2R:1G), three (2R:3G), or more green signals (the rearranged captured subtelomeric regions), previously described by Amiel et al [14]. Approximately 200 nuclei from each sample were analyzed and the slides were blindly scored.

**Telomere aggregate count**

As described in detail previously [16], aggregate size was divided into three groups relative to the size of a single telomere: 1) Fusion of 2–5 telomeres; 2) fusion of 6–10 telomeres; and 3) fusion of 11–15 telomeres. We used one filter for three colors, automatic exposure for both images, and ×100 magnification on an AX70 Olympus Provis microscope.

**Cytogenetic of random aneuploidy evaluation**

Random aneuploidy was visualized with the chromosome enumeration probes (CEP)-chromosome 18 Spectrum green (Catalog no. 5J10-18) and CEP chromosome 9 Spectrum orange (catalog no. 6J36-09) (Abbott Molecular-Vysis, Des Plaines, IL. USA). Probes for chromosomes 9 and 18 were used for detection of aneuploidy [14]. For each cell, we recorded the number of hybridization signals. The rate of aneuploidy was inferred from the fraction of cells revealing one, three or more hybridization signals per cell. Triploidy was defined as both probes (green and red signals) showing three signals in the same nucleus. At least 200 nuclei were scored from each sample and the slides were blindly scored.

**Statistical analysis**

A 2-tailed t test or nonparametric Mann-Whitney U test was applied to evaluate the differences between the groups, each when appropriate. Data were further analyzed by one-
way analysis of variance (ANOVA) or Kruskal-Wallis non-parametric test. Pearson correlation was used to assess correlation between shortened telomeres and survival in PSC.

Results

The study population included 20 patients in each of the following 3 groups: (1) IBD-group: 14 patients with UC and 6 patients with Crohn's disease (CD); (2) PSC group: 12 patients with UC, 3 with CD and 5 with no IBD; (3) 20 age- and sex-matched healthy controls. The mean age of the study cohort was 47.9 ± 7.1 and 43% were male. The demographic and clinical characteristics of each group are depicted in Table 1. There were no statistical differences in patient age, gender, BMI, smoking habit, glycated hemoglobin or duration of IBD among the study groups. However, patients with PSC-UC had significantly milder colitis activity than patients with UC only, as reflected by partial Mayo score (p = 0.001). All patients (100%) with PSC-UC had Mayo score grade 1, while only 35% of UC-patients had mild disease (p=0.003). Patients with concomitant PSC-CD also tended to have milder disease activity than patients with CD only, but this was not statistically significant (p=0.17).

Telomere length in the study population is depicted in Figure 1. At the lowest intensity, correlating with shorter telomeres, there were significantly more telomeres per cell in the lymphocytes from PSC patients than from IBD or healthy control patients (p=0.018 and < 0.001, respectively) while IBD patients had more than the controls (p < 0.001). At high intensity, denoting longer telomeres, the opposite picture was observed. Patients with PSC-IBD and PSC without IBD had the same rate of telomeres per cell at low and high intensity. We further assessed a possible correlation between the Mayo risk score and the percentage of cells with shortened telomeres in each patient (Figure 2). The range of cells with
shortened telomeres was 54-76% (average 65.15 ± 5.9) and no correlation was observed between the two variables (Correlation coefficient – 0.365, p = 0.113).

In order to calculate the rate telomere aggregates (TAs), we first established the baseline of TAs in the control group per case. These backgrounds TAs were considered on technical grounds due to a virtual overlap of telomeres that could not be distinguished under a two-dimensional (2D) microscope. There was significantly more fusion of 2-5 telomeres in the PSC group compared to the healthy control group (6.5 ± 3.4 and 2 ± 1.6, respectively, p=0.02), but not for fusion of > 5 telomeres (Figure 3). The IBD group was not different from healthy controls in all number-of-fusion subgroups. There was no difference between PSC-IBD and PSC without IBD in terms of number of cells with TAs (data not shown).

Telomere capture (TC) rate was compared between the groups as the number of the sub-telomeric regions (in signals). As shown in Table 2, the TC rate was significantly higher in the PSC and IBD groups for both probes, relative to the control group (p=0.01 and 0.04 for 15qter and 13qter probes, respectively). No difference in TC rates was noted between PSC-IBD and PSC without IBD patients (data not shown).

A significantly higher total rate of random aneuploidy (monosomy and trisomy) in both PSC and IBD groups compared to controls was found with the chromosome 9 probe (p= 0.04 and 0.001, respectively; table 3). In contrast, only the PSC group had a higher trisomy rate compared to IBD and control groups with the chromosome 18 probe, as well as triploidy (p<0.001). Similar to other parameters, no difference in total aneuploidy rates was noted among PSC patients with or without concomitant IBD.

Discussion

In the present study, we found the expression of several parameters of telomere dysfunction and genomic instability in two pre-malignant conditions, IBD and PSC, relative to a healthy control population. Peripheral blood lymphocytes from these patients were shown to have
shortened telomeres and higher rate of telomere aggregates (TAs), suggesting telomere
dysfunction which often appears in genomic instability conditions. While telomere
shortening is a non-specific phenomenon, signifying cellular ageing [8] as well as other
conditions with accumulated metabolic load [26], the co-existence of TAs and especially
random aneuploidy, indeed suggest genomic instability characteristic of a pre-malignant
state, as has been shown in other conditions as well [11-12, 17-18]. The lack of differences
among the study groups in factors with potential influence on telomere dysfunction, such as
patient age and major metabolic derangements (BMI, smoking habit, known diabetes), also
supports this point. We also demonstrated in the current and previous studies [21] that the
two suggested mechanisms for maintaining chromosomal integrity, i.e. telomere capture
(TC) and expression of telomerase (reflected by amplification of TERC), are also stimulated
in PSC and IBD patients. These mechanisms are mainly used by tumor cells [14, 27].

It was previously demonstrated that patients with UC have telomere shortening in
colonocytes, and that telomere shortening is associated with chromosomal instability and
anaphase bridges [28-29]. Shorter telomeres were associated with longer disease duration
[30], and since duration of disease is a main risk factor for developing malignancy in IBD,
this fits the notion that shortened telomeres can be a marker of pre-malignant potential.
Indeed, telomere shortening can be observed in the non-dysplastic epithelium adjacent to
dysplasia of UC patients, as part of a "field effect" and along with other chromosomal
instability parameters [31]. The telomere length of leukocytes, which travel through the
inflamed colon, was also significantly shorter in UC than in normal controls, although less
than in colonocytes, probably reflecting their decreased proliferative activity [30].

Similarly, telomere dysfunction has been described in several liver diseases involving
autoimmune or fibrotic processes [32]. Sasaki et al. demonstrated telomere dysfunction in
biliary epithelium of patients with primary biliary cirrhosis [33], and recently telomere
shortening was found in peripheral blood mononuclear cells of these patients, correlating with advanced disease [34]. The rate of fibrosis progression was also correlated with the rate of telomere shortening from liver biopsies of patients with hepatitis C virus [35], and shortened telomeres were actually observed in cirrhosis, irrespective of etiology [36].

It has been suggested that telomere dysfunction is implicated in immunosenescence phenomenon reflecting pre-mature ageing of immune system. Thus, many studies have observed that accelerated telomere loss is a common feature of systemic autoimmune disease, such as systemic lupus erythematosus or rheumatoid arthritis [37], and local or systemic fibrotic conditions, such as scleroderma [38], idiopathic pulmonary fibrosis [39] and cirrhosis [32, 36]. The combination of inflammatory, autoimmune and fibrotic processes in PSC, increases the potential for a telomere-telomerase system dysfunction, as found in our study. As described in age-related malignancies in lymphocytes of elderly cancer patients [40], it may also be that the worse immunosenescence observed in peripheral lymphocytes of PSC patients and induced by the autoimmune/fibrotic condition, may actually reflect the condition which facilitates the onset of malignancies in these patients, giving the fact that their malignant potential is not limited to the inflamed bile ducts.

In our study, most of the parameters of telomere dysfunction were more pronounced in PSC, which is known to be associated with a higher malignant potential than IBD without PSC. This was despite the fact that the severity of IBD, based on CRP blood level and symptom-based questionnaires, was more prominent in patients with IBD without PSC compared to PSC patients. Indeed, it is generally known that in patients with PSC, colitis tends to be mild or often only microscopic [3]. Moreover, the inflammation-induced malignant potential is by no means limited to the colon, but encompasses the entire hepato-biliary system, regardless of the severity of the inflamed colon. Lack of difference in the studied instability parameters between isolated PSC and PSC-IBD patients, supports this argument and corresponds with
evidence showing lack of difference between these groups in terms of prognosis and malignant potential. [41-42]

Although the numbers are small and reliable conclusions are difficult to be obtained, according to this cohort it seems that staging of disease, established by the Revised Mayo Score, is not reflected in the peripheral lymphocytes telomeres. It may be related to the fact that only 1 patient had Mayo Risk Score > 4, a suggested risk factor for cholangiocarcinoma [43]. Moreover, this model does not include duration of disease, a well-known factor in inflammation-carcinogenesis sequence, as one its parameters. However, 50% of cholangiocarcinomas are diagnosed within the first year of diagnosis of PSC [1], so contributing factors other than disease duration obviously play a role.

Another novelty of our research is that despite the fact that peripheral leukocytes are not the primary end target organ for PSC or IBD, these cells show telomere dysfunction. The appearance of shortened telomeres in peripheral immune system cells in epithelial malignancies can be seen as inability to control the ongoing carcinogenesis, being a potential prognostic marker for solid tumors [44]. In the context of PSC these parameters were not studied in the epithelial tissues, but aneuploidy, another pathognomonic marker of tumor genesis, is a well-known phenomenon in the evolution of PSC towards cholangiocarcinoma; Polysomy, tetrasomy and trisomy of chromosomes 7 and 3 were previously observed in bile duct cells from patients with PSC even before the development of cholangiocarcinima [45]. Since genetic instability is a continuous process influenced by the chronic inflammatory condition in the biliary tract, we assumed that what happens in biliary tissue is reflected in peripheral lymphocytes and can be used as a surrogate marker for the ongoing process, as leukocyte telomere length has been demonstrated to be correlating highly with that in cells from other tissues [20]. Alternatively, it may also be that the accumulating oxidative stress induced by the systemic inflammation is reflected in the telomeres of the peripheral immune
system, similar to what has been described in other systemic inflammatory conditions and metabolic derangements [26].

This study has some limitations. First, the small study groups preclude us from generalizing the results to all patients with PSC and IBD, because of the etiological, clinical and prognostic heterogeneity of the diseases, as well as the pre-malignant tendency. Clearly, larger studies encompassing more subgroups of patients are needed. Moreover, we studied telomeres in a single peripheral blood sample, at a single period, in patients with long-lasting chronic diseases. In order to better clarify the role of the telomere-telomerase system in the clinical progression of these inflammatory diseases and their potential evolution to malignancy, these patients should be followed prospectively with repeated samples reflecting their disease stage. Third, to get further insight, the diseased tissues (i.e. colon and biliary tract), which are the "target" tissues for malignancy, should also be sampled directly, in addition to blood lymphocytes, which only indirectly reflect the genetic changes in the chronically inflamed tissues.

Despite these shortcomings, this is the first study to demonstrate telomere dysfunction and random aneuploidy in peripheral blood lymphocytes from patients with PSC, which occur at a higher rate than in patients with IBD or healthy individuals. These findings attest to immunosenescence phenomenon and genetic instability, most likely reflecting the chronic autoimmune conditions and possibly their inflammation-induced malignant potential.
References


Figure 1: Telomere length: Mean number of cells in different subgroups of signal intensity (low, high)/telomere number (<10, 11≤30, >31) in the study population. Low and high signal intensity signify short and long telomeres, respectively.

* p value < 0.001 for Primary sclerosing cholangitis vs. healthy controls and 0.018 for Primary sclerosing cholangitis vs. Inflammatory bowel disease

**p value < 0.001 for Inflammatory bowel disease vs. healthy controls

***p value < 0.001 for healthy controls vs. Primary sclerosing cholangitis and Inflammatory bowel disease

PSC- primary sclerosing cholangitis; IBD- inflammatory bowel disease
Figure 2: A scatter plot demonstrating the correlation between the percentage of cells with shortened telomeres and Mayo risk score in patients with Primary sclerosing cholangitis. Each dot signifies one patient. The linear Pearson correlation coefficient (R) is 0.365 (p value – 0.113), suggesting lack of significant correlation between the two variables.
**Figure 3:** The mean number of cells with telomere aggregates in the study population.

*Statistical significance for Primary sclerosing cholangitis vs. healthy controls

PSC - primary sclerosing cholangitis; IBD - inflammatory bowel disease
Figure 2

Risk score vs. % cells with shortened telomeres
Figure 3

The bar chart illustrates the number of telomere aggregates across different categories of cells. The categories are labeled as 'Healthy', 'IBD', and 'PSC'. The chart shows the distribution of cells across different ranges of telomere aggregates:

- **1-5 telomere aggregates**:
  - Healthy: 7
  - IBD: 4
  - PSC: 3
  - P-value: 0.02

- **6-10 telomere aggregates**:
  - Healthy: 2
  - IBD: 3
  - PSC: 2
  - P-value: 0.3

- **11-15 telomere aggregates**:
  - Healthy: 0
  - IBD: 1
  - PSC: 1
  - P-value: 0.7
Table 1: Clinical characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Primary sclerosing cholangitis (n=20)</th>
<th>Inflammatory bowel disease (n=20)</th>
<th>Healthy (n=20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isolated (n=5)</td>
<td>With Ulcerative colitis (n=12)</td>
<td>With Crohn’s disease (n=3)</td>
<td>Ulcerative colitis (n=14)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.8 ± 7.8*</td>
<td>47.3 ± 8.4</td>
<td>47.5 ± 6.3</td>
<td>0.75</td>
</tr>
<tr>
<td>Gender N (% male)</td>
<td>9 (45)</td>
<td>8 (40)</td>
<td>9 (45)</td>
<td>0.38</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.1 ± 3.6</td>
<td>23.9 ± 4.4</td>
<td>23.6 ± 5.1</td>
<td>0.32</td>
</tr>
<tr>
<td>Smoking N (%)</td>
<td>3 (15)</td>
<td>3 (15)</td>
<td>4 (20)</td>
<td>0.22</td>
</tr>
<tr>
<td>Glycated hemoglobin</td>
<td>5.5 ± 0.4</td>
<td>5.7 ± 0.7</td>
<td>5.4 ± 0.5</td>
<td>0.35</td>
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<tr>
<td>Drugs N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Ursodeoxycholic acid</td>
<td>17 (85)</td>
<td>-</td>
<td>NR</td>
<td>&lt; 0.0001</td>
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<tr>
<td>- Mesalamine</td>
<td>12 (60)</td>
<td>15 (75)</td>
<td></td>
<td>0.311</td>
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<tr>
<td>- Thiopurines</td>
<td>2 (10)</td>
<td>10 (50)</td>
<td></td>
<td>0.014</td>
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<tr>
<td>Duration of Inflammatory bowel disease (years)</td>
<td>16.2 ± 5.5</td>
<td>13.9 ± 6.1</td>
<td>NR</td>
<td>0.38</td>
</tr>
<tr>
<td>Duration of Primary sclerosing cholangitis (years)</td>
<td>11.3 ± 3.4</td>
<td>NR</td>
<td>NR</td>
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<td>C-reactive protein level</td>
<td>0.7 ± 0.3</td>
<td>1.8 ± 0.9</td>
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<td>Mayo score</td>
<td>NR</td>
<td>1.1 ± 0.7</td>
<td>NR</td>
<td>4.1 ± 1.2</td>
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<td>Mayo score grade 1 N (% of patients)</td>
<td>NR</td>
<td>12 (100)</td>
<td>NR</td>
<td>5 (35)</td>
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<tr>
<td>Harvey-Bradshaw index</td>
<td>NR</td>
<td>NR</td>
<td>3.0 ± 1.0</td>
<td>NR</td>
</tr>
<tr>
<td>Crohn’s disease in remission/mild disease N (% of patients)</td>
<td>NR</td>
<td>NR</td>
<td>3 (100)</td>
<td>NR</td>
</tr>
</tbody>
</table>

* Unless specified otherwise, all values are presented as mean ± SD

NR- not relevant
### Table 2: The percentage of telomere capture (TC) signals from total number of nuclei in the study population (mean ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>15qter probe</th>
<th></th>
<th>13qter probe</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>2R:1G</td>
<td>2R:3G</td>
<td>2R:1G</td>
<td>2R:3G</td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>43.88 ± 6.14</td>
<td>7.74 ± 3.20</td>
<td>13.6 ± 3.89</td>
<td>5.03 ± 2.48</td>
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<td>Inflammatory bowel disease</td>
<td>47.48 ± 7.70</td>
<td>6.34 ± 3.87</td>
<td>11.89 ± 5.29</td>
<td>5.47 ± 3.22</td>
</tr>
<tr>
<td>Healthy</td>
<td>18.24 ± 3.74</td>
<td>4.85 ± 1.79</td>
<td>8.12 ± 5.91</td>
<td>3.36 ± 1.88</td>
</tr>
<tr>
<td>P value</td>
<td>0.001*</td>
<td>0.001*</td>
<td>0.001*</td>
<td>0.042*</td>
</tr>
</tbody>
</table>

*Statistical significance between PSC and IBD vs. healthy controls

PSC- primary sclerosing cholangitis; IBD- inflammatory bowel disease
Table 3: Random aneuploidy rates in the study population. Presented as percentage number of cells (mean ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Chromosome 9</th>
<th></th>
<th>Chromosome 18</th>
<th></th>
<th>Triploidy</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monosomy</td>
<td>Trisomy</td>
<td>Monosomy</td>
<td>Trisomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>11.81 ± 3.86</td>
<td>6.99 ± 3.39</td>
<td>19.44 ± 7.04</td>
<td>5.57 ± 3.40</td>
<td>1.75 ± 1.35</td>
<td></td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>11.28 ± 4.26</td>
<td>6.08 ± 4.40</td>
<td>18.25 ± 4.92</td>
<td>3.64 ± 1.76</td>
<td>0.87 ± 0.57</td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>8.54 ± 5.97</td>
<td>2.83 ± 2.34</td>
<td>9.55 ± 3.75</td>
<td>3.06 ± 1.89</td>
<td>0.39 ± 0.55</td>
<td></td>
</tr>
</tbody>
</table>

P value 0.042 * 0.001 * 0.001 * 0.006 ** 0.001 **

* Statistical significance for PSC and IBD vs. healthy controls

** Statistical significance for PSC vs. IBD and healthy controls

PSC- primary sclerosing cholangitis; IBD- inflammatory bowel disease