Peer Review File

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Response to comments by Reviewer 1

Comment 1: *In general, the text is well written but could use a thorough examination to remove typos and abbreviations/ full written words.*

Reply 1: We reexamined the whole text and corrected the abbreviations/full text as below. We also used spell check function of Word.

Changes in the text:

- According to the Surveillance, Epidemiology, and End Results database, histopathological diagnoses other than adenocarcinoma were more frequent in patients aged 15–19 (26.7%) than in those aged 35–39 (13.6%) (46). (page 8, line 18–page 9, line 1)
- They found that mutations in *MSH2* and *MSH6*, as well as the microsatellite instability (MSI)-high phenotype, were more frequent in right-sided AYA-CRC than in left-sided AYA-CRC (20.8% vs. 4.8%); clinical significance of *MSH2*/*MSH6* mutations and MSI-high phenotype is described in the following section. (page 9, line 9–13)
- Physicians who treat AYA-CRC patients should also explain fertility preservation to patients before treatment. (page 15, line 16–18)

Comment 2: *search string: "((adolescent and young adult) OR AYA) AND (colorectal cancer)" and (("young-onset AND (colorectal cancer)". What about adolescent or young (adult), or Colon cancer/rectal cancer, early onset (not only "young-onset")? I believe the entire search string should be included.*

Reply 2: We think that the reviewer's suggestion is very important. However, if we used the search strings proposed by the reviewer, over 8,000 articles were to be analyzed and most of them do not focus on the AYA population. Since this article is a narrative review and not a systematic review, we think more simple search strings will be acceptable. We modified the search strings as below, and added some important references related to early-onset CRC.

Changes in the text:

- We searched the articles published in the PubMed database until November 30, 2022, with keywords, "((adolescent and young adult) OR AYA) AND ((colorectal cancer) OR (colon cancer) OR (rectal cancer))" and "young-onset AND ((colorectal cancer) OR (colon cancer) OR (rectal cancer))". (Abstract)
- Although there have been a lot of excellent review articles about young-onset CRC (10–12), most of them do not focus on the AYA population. (page 4, line 19–page 5, line 1)
- Articles related to AYA-CRC were identified using keywords, "((adolescent and young adult) OR AYA) AND ((colorectal cancer) OR (colon cancer) OR (rectal cancer))." We also searched and reviewed articles related to young-onset CRC using keywords such as "young-onset AND ((colorectal cancer) OR (colon cancer) OR (rectal cancer))." (page 5, line 8–11)
- In a large cohort of female nurses aged 25–42 years, higher intake of vitamin D was associated with decreased risk of early-onset CRC; thus, vitamin D intake may be encouraged in young women (44). (page 8, line 7–9)

- The consensus molecular subtype (CMS) is a recently developed classification system for CRC at the gene expression level (54), and it has been reported that CMS1 was the most common in the AYA age group (55). (page 9, line 17–19)
- Pearlman, et al. have analyzed the prevalence of germline mutations associated with cancer susceptibility among 450 CRC patients younger than 50 years, and they revealed that 16% of patients had genetic cancer susceptibility (60). (page 10, line 11–13)
- Table 1 and Figure 1 were revised accordingly.

Comment 3: *There are other quite similar reviews, and I would suggest emphasizing the focus of this review and what makes it different to other recent reviews (from other countries).*

Reply 3: We think that the strength of our review lies in that we focused on AYA age group, while most previous reviews focused on wider population (e.g., aged <50 years), and that we described local situations in Japan. We added the sentences below to explain such strength.

Changes in the text:

- Although there have been a lot of excellent review articles about young-onset CRC (10–12), most of them do not focus on the AYA population (15–39 years old). As far as we know, this is the first review article that highlights current situations of AYA-CRC in Japan. (page 4, line 19–page 5, line 3)

Comment 4: *Line 55 - Colorectal cancer (CRC) is the most common cancer. I believe it is breast cancer and CRC the second.*

Reply 4: As the reviewer pointed out, breast cancer is the most common cancer worldwide. However, when it comes to the Japanese statistics, colorectal cancer is more common than breast cancer. We revised the sentence as below.

Changes in the text:

- Among AYA individuals in Japan, CRC is the fourth most common cancer after breast, uterine, and thyroid cancers (3). (page 4, line 6–7)

Comment 5: *Line 87 - "There are no reports on the global epidemiology of AYA-CRC". I disagree. Please see: ahttps://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-022-14274-7.*

Reply 5: We have deleted the original sentence and added the sentence below.

Changes in the text:

- The Global Burden of Disease Study has reported the global epidemiology of AYA-CRC; the global incidence of AYA-CRC had increased from 37,285 in 1990 to 76,090 in 2019 (22). (page 6, line 4–8)

Comment 6: *Line 106: I would suggest replacing race with another word. For example, genetic ancestry.*

Reply 6: We revised the expression from "race" to "genetic ancestry".

Changes in the text:

- Disease stage at diagnosis is the most striking prognostic factor, whereas male sex, black genetic ancestry, no insurance, poorly differentiated histology, higher tumor grade, and exposure to fine particulate matter pollution have also been reported to be associated with worse survival (33–35). (page 7, line 3–6)

- On the other hand, two studies assessed the risk factors for CRC or rectal cancer in AYAs and revealed that family history of CRC, genetic ancestry other than black or white, and inflammatory bowel disease were associated with a higher frequency of AYA-CRC (42,43). (page 8, line 1–4)

Comment 7: *Lynch syndrome, MSI status (core MMR proteins are mentioned before they are introduced. It would improve the manuscript if they were introduced first or a referral to the later Lynch section.*

Reply 7: We revised the sentence as below.

Changes in the text:

- They found that mutations in *MSH2* and *MSH6*, as well as the microsatellite instability (MSI)-high phenotype, were more frequent in right-sided AYA-CRC than in left-sided AYA-CRC (20.8% vs. 4.8%); clinical significance of *MSH2*/*MSH6* mutations and MSI-high phenotype is described in the following section. (page 9, line 9–13)

Comment 8: *MSI should be written out first time it is used.*

Reply 8: We revised it accordingly.

Changes in the text:

- They found that mutations in *MSH2* and *MSH6*, as well as the microsatellite instability (MSI)-high phenotype, were more frequent in right-sided AYA-CRC than in left-sided AYA-CRC (20.8% vs. 4.8%); clinical significance of *MSH2*/*MSH6* mutations and MSI-high phenotype is described in the following section. (page 9, line 9–13)

Comment 9: *Line 169-170 "methylation of MLH1 can be other causes of Lynch syndrome". Incorrect. Sporadic methylation of MLH1 is not Lynch. Please correct.*

Reply 9: We revised the sentence as below.

Changes in the text:

- Hypermethylation of *MLH1* can cause sporadic dMMR/MSI-high CRC, but this condition is not considered Lynch syndrome. (page 11, line 8–10)

Comment 10: *Line 172 Pold is also not considered Lynch.*

Reply 10: We did not intend to describe *POLD1* mutation as a cause of Lynch syndrome. We revised the sentence as below.

Changes in the text:

- Two teenage siblings with multiple adenomas and CRC have been reported who had heterozygous variants in *PMS2* and *POLD1* (57). *POLD1* mutations are also known as a cause of hereditary CRC (64); thus, the coexistence of these variants may have accelerated cancer predisposition. (page 11, line 12–15)

Comment 11: *I would suggest introducing MMR, dMMR caused by Lynch, and BRAF v600e / MLH1 promoter methylation-related dMMR.*

Reply 11: We added the explanation about MMR, dMMR, *BRAF* V600E mutations, and *MLH1* promoter methylation as below.

Changes in the text:

- These mismatch repair proteins correct DNA replication errors and they are essential to maintain genetic stability (63). In Lynch syndrome, mismatch repair deficiency (dMMR) accelerates mutation accumulation and increases the risk of carcinogenesis. dMMR-associated cancer usually shows high tumor mutation burden and MSI-high phenotype (63). (page 11, line 3–7)
- Hypermethylation of *MLH1* can cause sporadic dMMR/MSI-high CRC, but this condition is not considered Lynch syndrome. (page 11, line 8–10)
- Mutations in BRAF are found in up to 12% of metastatic CRC and BRAF V600E mutations are associated with the female sex, right-sided and advanced CRC, and high mutation burden (68), but these mutations are rare in patients with Lynch syndrome; thus, BRAF-specific immunohistochemistry may be useful to exclude Lynch syndrome (69). (page 12, line 1–4)

Comment 12: *Line 173-176 – the revised Amsterdam criteria should be mentioned.*

Reply 12: We added a sentence about the revised Amsterdam criteria as below.

Changes in the text:

- The Amsterdam criteria has been revised in 1999 (66); however, its mutation detection rate was not improved enough compared to the original criteria (67). (page 11, line 18–page 12, line 1)

Comment 13: *Line 182 microsatellite instability is used here instead of MSI.*

Reply 13: We revised the words accordingly.

Changes in the text:

- They found that mutations in *MSH2* and *MSH6*, as well as the microsatellite instability (MSI)-high phenotype, were more frequent in right-sided AYA-CRC than in left-sided AYA-CRC (20.8% vs. 4.8%); clinical significance of *MSH2*/*MSH6* mutations and MSI-high phenotype is described in the following section. (page 9, line 9–13)

Comment 14: *Line 247 Colorectal cancer is used instead of CRC.*

Reply 14: We revised the words accordingly.

Changes in the text:

- Physicians who treat AYA-CRC patients should also explain fertility preservation to patients before treatment. (page 15, line 16–18)

Response to comments by Reviewer 2

Comment 1: *Study also missing huge population cohort 40-49y old.*

Reply 1: There have been many original and review articles about young-onset CRC including the 40–49 years age group. However, we intended to focus on AYA age group in this review, and we did not review all articles about young-onset CRC (e.g., <50 years old). We added an explanation why we focused on AYA age group, and added some important references on young-onset CRC.

Changes in the text:

- Although there have been a lot of excellent review articles about young-onset CRC (10–12), most of them do not focus on the AYA population. As far as we know, this is the first review article that highlights current situations of AYA-CRC in Japan. (page 4, line 19–page 5, line 3)
- In a large cohort of female nurses aged 25–42 years, higher intake of vitamin D was associated with decreased risk of early-onset CRC; thus, vitamin D intake may be encouraged in young women (44). (page 8, line 7–9)
- Pearlman, et al. have analyzed the prevalence of germline mutations associated with cancer susceptibility among 450 CRC patients younger than 50 years, and they revealed that 16% of patients had genetic cancer susceptibility (60). (page 10, line 11–13)

Comment 2: *Gender specificity is missing at some places in the manuscript.*

Reply 2: We added some sentences related gender specificity as below.

Changes in the text:

- A multinational cohort study in Asia showed that the increasing trend of young-onset (including 40–49 age group) was the most pronounced in male rectal cancer (28). (page 6, line 14–15)
- In a large cohort of female nurses aged 25–42 years, higher intake of vitamin D was associated with decreased risk of early-onset CRC; thus, vitamin D intake may be encouraged in young women (44). (page 8, line 7–9)
- Especially, male patients aged <50 years were reported to have a higher risk of mental health disorders after diagnosis of CRC compared to average-age CRC patients (98). (page 17, line 1–3)

Comment 3: *In Epidemiology explaining more about life style modifications would help.*

Reply 3: We added a sentence as below.

Changes in the text:

- Recent changes in lifestyles such as Western-style diet and physical inactivity are thought to have caused the increase of the incidence of AYA-CRC (23). (page 6, line 6–8)

Comment 4: *Would recommend more descriptive concise narration in Hereditary cancers.*

Reply 4:

Changes in the text:

- While 3–5% of these have well-characterized hereditary cancer syndromes such as Lynch syndrome, familial adenomatous polyposis (FAP) and other rare syndromes <u>including MutYH-associated polyposis, Peutz-Jeghers syndrome, juvenile polyposis, polymerase proofreading-associated polyposis and Cowden/PTEN hamartoma syndrome</u> (Table 3), while other hereditary cases remain unexplained (57,58). (page 10, line 4–8)
- In most hereditary CRC syndromes, polyps undergo carcinogenesis, but the exact route to carcinoma seems to differ between the conditions (59). Pearlman, et al. have analyzed the prevalence of germline mutations associated with cancer susceptibility among 450 CRC patients younger than 50 years, and they revealed that 16% of patients had genetic cancer susceptibility (60). (page 10, line 9–13)

Comment 5: *Duplications and contradictory lines found- eg. Line 57 and Line 261.*

Reply 5: In line 57, we explained the order of the incidences of each site of cancer. On the other hand, in line 261, we described which site of cancer is common as a secondary cancer after diagnosis of AYA-CRC. These sentences

are not duplications.

Changes in the text:

- (No changes)

Comment 6: *Reference articles hypothesis not clearly delineated. eg- Salem et al study not in AYA.*

Reply 6: Basically, we searched for articles about AYA-CRC. The study by Salem, et al. is also about AYA-CRC; thus, we revised the sentence as below. We also quoted some articles about young-onset CRC (e.g., including 40–49 age group) in this review, but in such cases, we clearly described the age ranges of target populations.

Changes in the text:

- Salem et al. compared the molecular profiles of right- and left-sided CRC in AYAs (50). They found that mutations in *MSH2* and *MSH6*, as well as the microsatellite instability (MSI)-high phenotype, were more frequent in right-sided AYA-CRC than in left-sided AYA-CRC (20.8% vs. 4.8%); clinical significance of *MSH2*/*MSH6* mutations and MSI-high phenotype is described in the following section. (page 9, line 8–13)