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Age and Gender Disparities in Cancer Biomarker Expression: Insights from a Population-Based Investigation in Mymensingh District of Bangladesh

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ABSTRACT

Cancer remains a significant global health challenge, necessitating innovative approaches for early detection and targeted therapeutic interventions. To revolutionize cancer therapy by rapid identification and evaluation of biomarkers, this study set out to ascertain the frequency of cancer markers in Bangladesh's north-central area. A cross-sectional investigation encompassing 463 individuals was conducted between March 2021 and July 2023. The detection of serum cancer biomarkers was accomplished by using an ELISA-based chemiluminescent microparticle immune assay. The independent variables were compared using an unpaired t-test. Five categories were created from the data clustering: Healthy control, group 1 (10-17 years, adolescent), group 2 (18 to 35 years, younger adult), group 3 (36 to 55 years, middle aged), and group 4 (>55 years, older adult). The serum level of PSA (prostate-specific antigen) was greater in group 4 compared to healthy control group (8.011 ± 1.281 ng/ml vs 1.307 ± 0.1598 ng/ml). The serum level of CA-125 (Carbohydrate Antigen-125) was greater in group 3 and group 4 compared to healthy control group (65.70 ± 6.430 vs 16.57 ± 0.793 and 1045 ± 275.8 vs 16.57 ± 0.793, respectively). The carcinoembryonic antigen (CEA) was expressed more often in group 4 male and female participants compared to the healthy controls (8.423 ± 0.5059 vs 2.436 ± 0.1086). Alpha-feto protein (AFP) was expressed more often in male group 3 and group 4 compared to healthy control group (15.24± 6.823 vs 4.615 ± 0.6613 and 18.54 ± 5.757 vs 4.615 ± 0.6613) and Male individuals reported a considerably greater amount of AFP than female individuals. This study revealed that older individuals had elevated levels of PSA, CA-125, CEA, and AFP. Moreover, males had greater levels of AFP than females.

1. Introduction

The significant rise of cancer cases is one of the major reasons for illness and fatality in Bangladesh as well as the other parts of the world. Worryingly, by 2040, there will be an estimated 28.4 million cases worldwide of cancer, a 47% increase from 2020 (Sung et al. 2021). Additionally, in terms of fatalities, ischemic heart disease is the leading cause death globally (8.97 million fatalities) followed by cancer, but cancer is expected to overtake it in 2060 (~18.63 million fatalities) (Mattiuzzi et al. 2019), and in Bangladesh, it is also the sixth largest reason of mortality, with 66% of cancer patients being between the ages of 30 and 65, which is nonetheless the major working people worldwide (Hussain et al. 2013; Fitzmaurice et al. 2017). Even if the incidence of risk factors differs by location and nation, they are essentially comparable worldwide (Aune et al. 2016). Considering the risk factors, it is crucial to carry out prompt identification, as this is the main objective for effective supervision of cancer treatment as well as to give individuals a meaningful life expectancy.

A feature that may be determined to indicate cancer risk, prevalence, or patient prognosis is called a cancer biomarker. These features may be imaging-based, cellular, molecular, or physiological in nature. These substances are created by cancer cells or by ordinary cells in anticipation of malignancy and can be discovered in tissues or bodily fluids (Sarhadi et al. 2022). Several protein-based markers have previously been utilized for the diagnosis of cancer, such as prostate-specific antigen (PSA) for prostate cancer [Ilic et al. 2018], carbohydrate antigen 125 (CA-125) for ovarian cancer (Charkhchi et al. 2020), carcinoembryonic antigen (CEA) for colorectal cancer (Campos-da-Paz et al. 2018), alpha-fetoprotein (AFP) for liver cancer (Zhang et al. 2020) and so on.

Since each biomarker functions inside the body and acts uniquely to therapeutic regimens, they ultimately help doctors to plan treatment strategies. The fact that cancer yet seems to be difficult to cure may be attributed to late-stage diagnosis, whereas early-stage diagnosis has long been a source of apprehension in the research field.In

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addition, diagnostic evidence and the fluctuating character of cancer biomarkers that arise at a specific demographic level are crucial for determining the prediction of cancer. Because of the dearth of available information, the potential situation of different cancer biomarkers from Bangladesh, including PSA, CA-125, CEA and AFP remain unclear. Furthermore, it is still unknown how and to what degree the presence of these cancer biomarkers is related to age and sex in Bangladeshi population.

2. Materials and Methods

2.1. Study design and data collection

From March 2021 to July 2023, 463 individuals' (Male: 231 individuals and Female: 232 individuals) medical information was obtained from Popular Medical Service Centre in north-central region (Mymensingh district) in Bangladesh. This population-centered cross-sectional study gathered information on a number of cancer biomarkers, including PSA, CA-125, AFP, and CEA, which were advised by the local doctors. Medical information (such as age, sex, date, and values of corresponding cancer biomarkers) was extracted from the Popular Medical Service Centre according to the correct procedures. A total of 463 individuals were enrolled in this study. Among them, there were 120 individuals in the healthy control group, with each biomarker containing a subgroup of 30 healthy individuals. In case of AFP, the number of the study individuals in each group were: Healthy controls= 30 individuals, Group 1= None, Group 2= 20 individuals, Group 3= 35 individuals and Group 4= 45 individuals. In case of CA-125, the number of the study individuals in each group were: Healthy controls= 30 individuals, Group 1= 3 individuals, Group 2= 38 individuals, Group 3= 30 individuals and Group 4= 15 individuals. In case of CEA, the number of the study individuals in each group were: Healthy controls= 30 individuals, Group 1= None, Group 2= 13 individuals, group 3= 41 individuals and group 4= 32 individuals. In case of PSA, the number of the study individuals in each group were: Healthy controls= 30 individuals, Group 1= None, group 2= 1 individuals, group 3= 14 individuals and group 4= 56 individuals.

2.2. Chemiluminescent microparticle immune assay based on ELISA

In accordance withthe producer's instructions, a chemiluminescent microparticle immunological assay was employed to measure the degree of expression of each putative cancer biomarker (ARCHITECT i1000SR immunoassay analyzer, Abbott, USA). Whole blood from the individual was drawn into a serum separator container (BD Vacutainer® SST™, Georgia,United States) and serum was separated by centrifuging at 1500×g for 15 minutes at ambient temperature after being left to clot for 30 minutes. An established standardization process was employed to ensure the accuracy of each/daily runs, and 100–150 µl of serum wasutilized for every specimen. Leveraging chemiluminescent microparticle immunoassay (CMIA) technology, this test is an automated two-step immunoassay to identify the existence of certain biomarkers in human serum, plasma, and amniotic fluid. The initial stage involves combining the specimen, assay diluent, and anti-biomarker covered paramagnetic microparticles. After washing, the intended biomarker or antigen in the specimen adheres to the initial anti-biomarker-coated microparticles. Next, an additional anti-biomarker with an acridinium tag is incorporated as a conjugate. The reaction mixture is then supplemented with the pre-trigger (1.32% hydrogen peroxide, w/v) and trigger solutions (0.35N sodium hydroxide). The ARCHITECT immune assay optical system measures the chemiluminescent response as relative light units (RLUs), which correspond to the quantity of biomarker available in the specimen. For PSA, CA-125, AFP, CEA, and, the reference numbers of the ARCHITECT reagent kit that were utilized are 7K71, 2K45, 7K67, and 7K68 correspondingly (Abbott Laboratories, Abbott Park, Illinois, USA).

2.3. Clinical data analysis

To analyze diagnostic information, individuals were divided into five categories that were specified, for instance- Healthy controls, group 1 (10-17 years, adolescent), group 2 (18 to 35 years, young adult), group 3 (36 to 55 years, middle), and group 4 (>55 years, older adult). Analysis was performed as well on sex-related cancer risk.

2.4. Statistical analysis

A multivariate statistical tool, GraphPad Prism 9 software (San Diego, CA, USA) was used to analyze all of the obtained data sets.p-values <0.05 and < 0.005 are denoted by an asterisk (significant) and double asterisk (significant) respectively, whereas p-values > 0.05 are denoted by ns (not significant). To evaluate the variances between the explanatory variables, unpaired two-tailed t-test were used.

3. Results

3.1. Characteristic features of the studied population

As per the local physicians/medical professional's recommendation or suspicion of malignancy, all feasible clinical information sets were gathered from 2021 to 2023 and those are the prostate-specific antigen (PSA), carbohydrate antigen 125 (CA-125), alpha-fetoprotein (AFP), and carcinoembryonic antigen (CEA) for prostate, ovarian, liver, and colon cancer correspondingly. In order to determine the frequency of cancer biomarkers within this particular region relying on individual age and sex variance, an overall group of n=463 was investigated. Among the four indicators, PSA is an indicator unique to male (Ilic et al. 2018), whereas CA-125 is an indicator particular to female (Charkhchi et al. 2020). The remaining two indicators pertain to both male and female. Those individuals who contain standard amount of serum biomarkers are considered as healthy controls.

The rest of the independent groups that investigated the distribution of cancer biomarkers in both males and females across the age groups of adolescents, adults, middle age, and older adults were compared with healthy controls group. 50.11% of individuals were men and 49.89% were female.Ovarian and liver malignancy indicators, with rates of 32.18% and 26.13%, correspondingly, were more prevalent among individuals taking prescription.Contrarily, the colon and prostrate cancers have a corresponding prevalence of 22.46% and 19.22% (Figure 1: A-B).

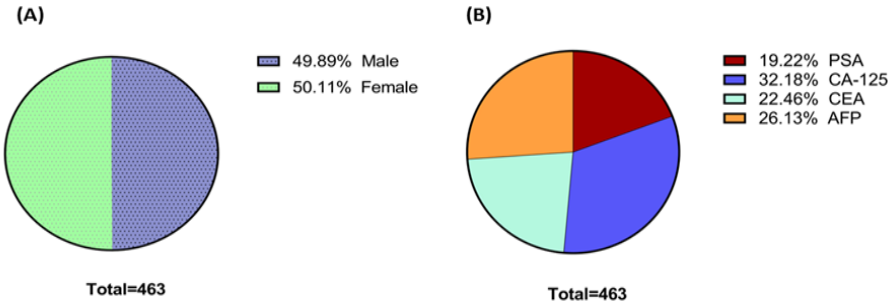

Figure 1. (A): Percentage of male and female individuals. (B) Percentages of investigated biomarkers (PSA, CA-125, CEA, AFP).

3.2. Gender associated cancer biomarkers

3.2.1. Prostate Specific Antigen (PSA)

The amount of serum PSA expression (ref value, 0–4 ng/ml) appears to be greater in the group 4 (8.011 ± 1.281 ng/ml) notwithstanding the fact that reached significantly with the healthy controls (1.307 ± 0.1598ng/ml), on the other hand, group 2 (0.010 ± 0 ng/ml) did not reach significantly with the healthy controls. Group 1 data wasn't available for PSA. Moreover, group 3 (0.4079 ± 0.0343 ng/ml) expression levels showed statistical significance with the healthy controls (Figure 2). Our analysis showed that PSA is upregulated in group 4 and they may have a greater risk of experiencing prostate-related complications.

Figure 2. The blood PSA expression level is higher in group 4 participants (Older adults) compared to the other groups; however, this is statistically significant with healthy controls, ****p <0.0001, ns stands for not significant, utilizing unpaired t-test. Moreover, group 3 individuals also (Middle aged people) showed statistical significance, **p<0.01(p=0.0038) with the healthy controls. Data represent mean.

3.2.2. Carbohydrate antigen 125 (CA-125)

CA-125 is a type of cancer biomarker which is utilized for determining ovarian cancer. The amount of serum CA-125 expression (ref value, 0-35 U/ml) seems significantly greater in in group 3 (65.70 ± 6.430) and group 4 (1045 ± 275.8) than the healthy controls (16.57 ± 0.793) (Fig. 3). Our analysis showed that CA-125 is upregulated in group 3 and group 4 which ultimately led to the greater risk of experiencing ovarian cancer.

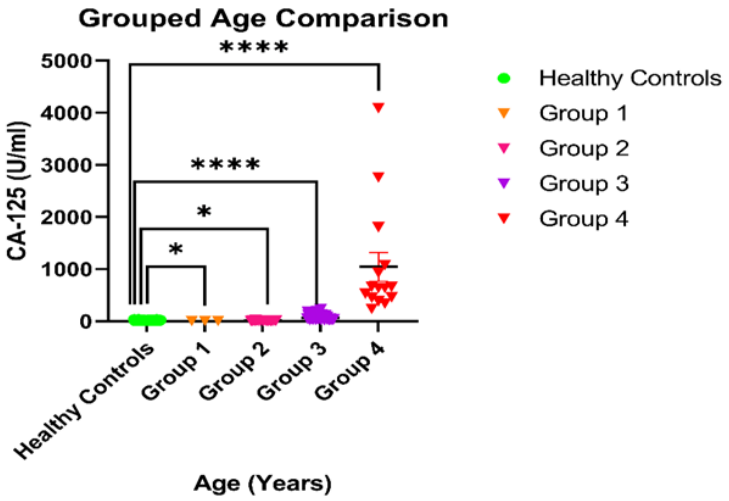

Figure 3. The blood CA-125 expression level is higher in group 3(Middle aged) and group 4 (Older adults) participants compared to the other groups and showed statistical significance with healthy controls, ****p <0.0001, utilizingunpaired two tailed t-test. Data represent mean±SEM.

3.3. Cancer biomarkers common for both genders

3.3.1. Carcinoembryonic antigen (CEA)

In order to identify and treat cancer of the large intestine and rectum, a carcinoembryonic antigen (CEA) test is leveraged. Typically, healthy individuals' blood contains 2-4 ng/mL of CEA, which is found inhighly modest amounts. The amount of serum CEA expression was significantly greater in the group 4 (8.423 ± 0.5059) participants compared to the healthy controls (2.436 ± 0.1086) (Figure 4-A).

Additionally, we tried to find out any significant changes in the amount of CEA in males and females in comparison to healthy controls group respectively (Figure 4-B and 4-C). Interestingly, we found statistically significance in the amount of CEA in males and females of group 4. Male individuals reported a considerably greater amount of CEA than female individuals when we regrouped the the data sets according to gender disparity, indicating that men may be more prone to cancer of the large intestine and rectum (Figure 4-D).

3.3.2. Alpha-feto protein (AFP)

For the identification of hepatocellular carcinoma, the blood biomarker alpha-feto protein (AFP) is well recognized when applied with ultrasonography along with various methods of imaging [Hanif et al. 2022]. The amount of serum AFP expression (ref value, 0-40 ng/mL) seems significantly greater in group 3 (middle-aged) and group 4 (Older adults). There were notable differences between group 4 (18.54 ± 5.757) and healthy controls (4.615 ± 0.6613) and even between group 3 (15.24 ± 6.823) and healthy controls ((4.615 ± 0.6613) according to our assessment (Figure 5-A).

Additionally, we tried to find out any significant changes in the amount of AFP in males and females in comparison to healthy controls group respectively (Figure 5-B and 5-C). Male individuals reported a considerably greater amount of AFP than female individuals when we regrouped the data sets according to gender disparity, indicating that men may be more prone to developing liver-related complications (Figure 5-D).

4. Discussion

Cancer biomarkers are employed in clinical practice for a variety of objectives, including risk assessment, differential diagnosis, distinguishing the benign or malignant character of tumors, diagnosis and prevention, assessing prognosis, response to therapy, and recurrence. Future cancer medicines have difficulty in implementing biomarkers to detect and treat the condition before it manifests itself through other extensive tests or even through symptoms. Cancer screening is gaining preeminence among the general people. The timely determination of biomarkers particular to a certain cancer is essential for both early identification of cancer and successful treatment outcomes [Kamali et al. 2022]. Determining cancer biomarkers in the localized region helps the doctor create a treatment strategy that not only lowers mortality as well as morbidity but also saves lives. Individuals who are diagnosed with these biomarkers early have a 92% chance of surviving, compared to 25% for those who are diagnosed with those late or after metastasis [Safi et al. 1991].

We selected these tumor markers for screening because we were interested in detecting possible cancer indicators thatarenot yet established all over Bangladesh specifically in the district or countryside area as a standard screening method. In our study, the information was divided into several distinct groups.

Individuals whose age are >55 years had a greater serum PSA, CA-125 and CEA concentration compared to the healthy control groups. CEA levels were significantly

higher in male than female participants. Middle aged participants had higher serum AFP levels compared to others.

Prostate cancer is a problem amongthe elderly, according to earlier research, and our analysis found that persons over 50 had higher serum levels ofPSA, corroborating this claim (Hussein et al. 2015).

According to research conducted in Sweden with 5550 female who had CA-125 screening, the author observed that female over 50 years of age had higher levels of CA-125, which correlates with the findings of our study (Einhorn et al. 1992).

Hepatocellular carcinoma (HCC) has a raised AFP level, and early identification is highly preferred since patients with early stages are typically asymptomatic and commonly discovered at the late stage when it is incurable (Mittal et al. 2011).According to reports, there is a substantial relationship between the dimensions of the tumor and AFP, and males are more likely than female to have HCC (Abbasi et al. 2012; McGlynn et al. 2015).

The older adult group 4 in our datasets had greater CEA expression levels in both males and females, supporting a previous study that found a correlation between higher CEA levels and advanced age, high body mass index, and poorly managed diabetes (Liu et al. 2022).Blood CEA is a useful non-intrusive blood test that is frequently used to track the efficacy of chemotherapy or radiation therapy for individuals with colorectal cancer (Knychalski et al. 2012; Gobbi et al. 2008).

Recently, a study was conducted in the northeastern part

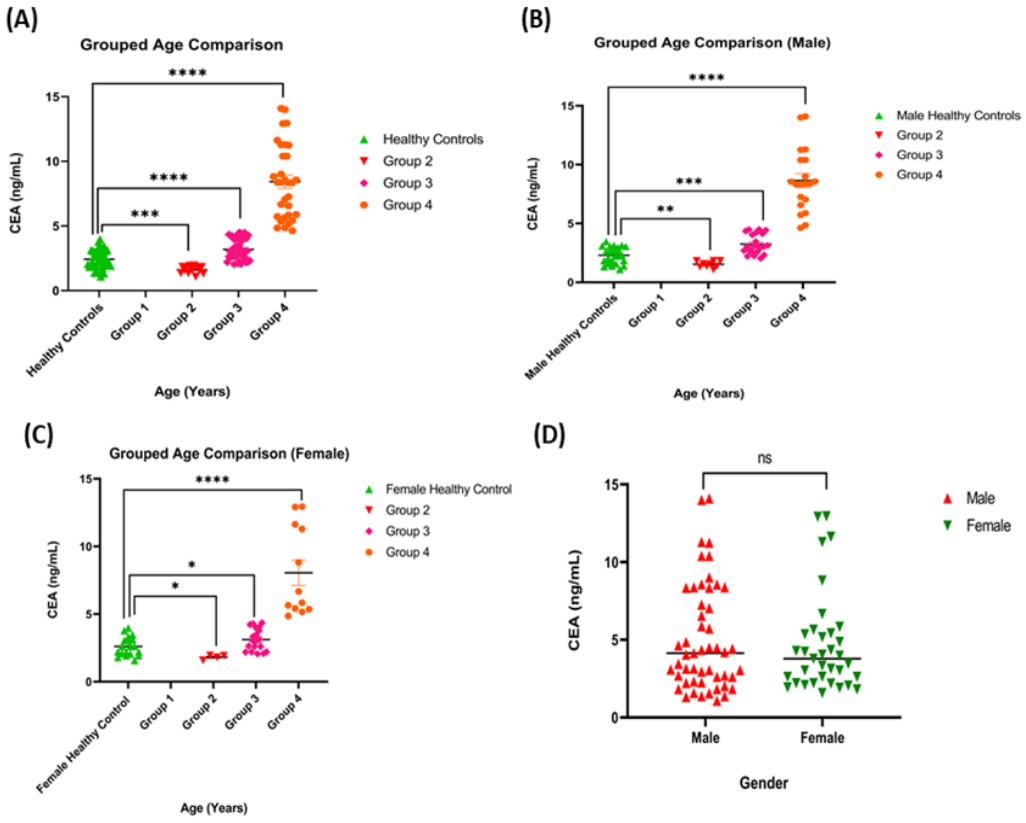

Figure 4. (A) The blood Carcinoembryonic antigen (CEA) expression level is higher in group 4 participants (Older adults) compared to the other groups; It is statistically significant with healthy controls, ****p <0.0001, utilizing unpaired t-test. Group 2 and group 3 individuals (Young adult and middle-aged people) showed statistical significance, ***p <0.001 (p= 0.0002) and ****p <0.0001 with the healthy controls respectively. We have no data of Carcinoembryonic antigen (CEA) for group 1. (B) The blood Carcinoembryonic antigen (CEA) expression level is higher in group 4 male participants (Older adults) compared to the other groups and was statistically significant (****p <0.0001) compared to healthy controls. Group 2 and group 3 were also statistically significant, **p (p= 0.0036) and ***p (p= 0.0001) respectively compared to healthy controls. (C) The blood Carcinoembryonic antigen (CEA) expression level is higher in group 4 female participants (Older adults) compared to the other groups and was statistically significant (****p <0.0001) compared to healthy controls. Group 2 and group 3 were also statistically significant, *p < 0.05 (p= 0.0368) and *p < 0.05 (p= 0.0456) respectively compared the healthy controls. (D) No statistical significant difference was observed in gender disparity in any age group of patients, ns stands for not significant, using a two-tailed unpaired student's t-test. Data represent mean±SEM.

of Bangladesh where researchers found that in case of PSA, prostate cancer is more common in older individuals (group 4) than in middle-aged individuals (group 3). This suggests that prostate cancer is a disease that primarily affects older individuals (Hasnat et al. 2022). This study also showed that a genealogy of breast or ovarian cancer, post menopause, hormonal disorders, overweight, and oral contraceptives are among a handful of variables that have been linked to ovarian cancer in elderly females. The higher incidence of CA-125 in middle-aged and elderly females from this relatively small population is extremely concerning (Hasnat et al. 2022).

Our present study has several limitations, and one of the most significant ones was that the medical facility may have had a policy not to divulge to us whether the patients were taking medication at the time of enrollment or what stage of cancer they had.

Additionally, just one tertiary hospital served as the site for this investigation. These restrictions may have an impact on the study's overall variable distribution, the relationships that were found, and the generalizability of its findings.

Future initiatives ought to focus on using a variety of tumor markers to screen for cancer. It is necessary to investigate these findings in additional suburbs in addition to other urban cities.

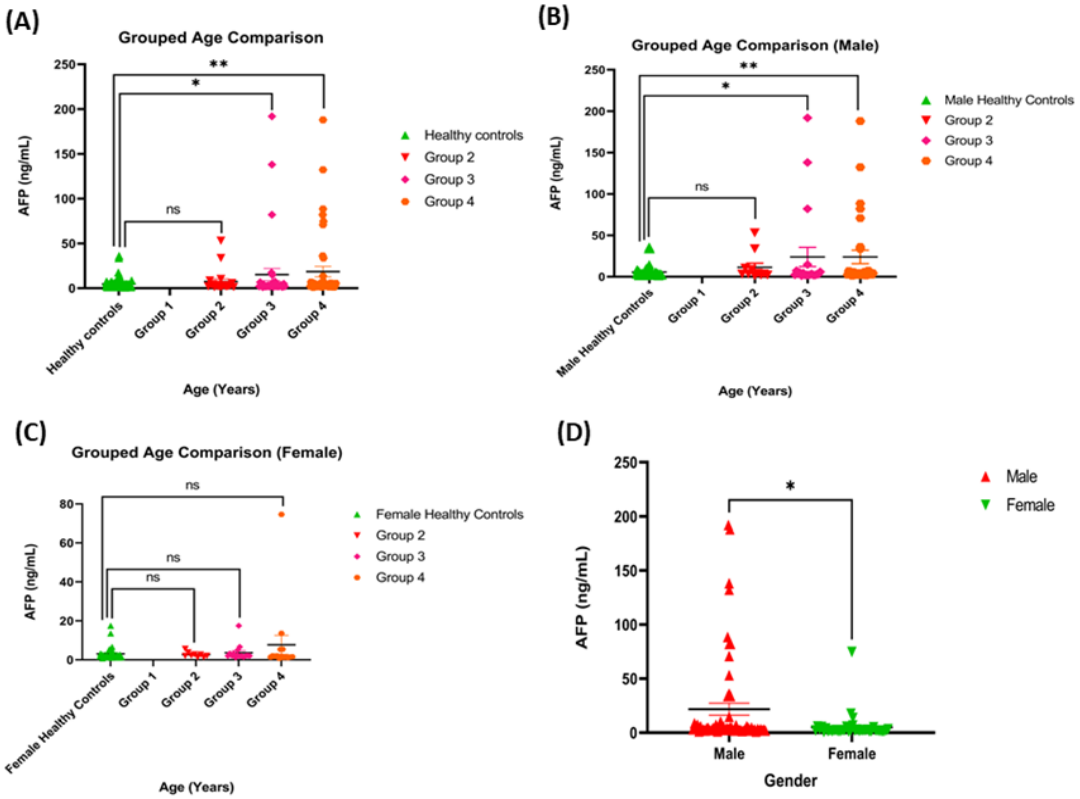

Figure 5. (A) The blood Alpha-feto protein (AFP) expression level is higher in group 3 (middle-aged) and group 4 (Older adults) participants compared to the other groups; group 4 showed that they are statistically significant with the healthy controls, **p (p= 0.0011). Group 3 individuals (middle-aged people) also showed statistical significance, *p (p= 0.0162) with the healthy controls. Utilizing unpaired two tailed t-test. We have no data of AFP for group 1. (B) The serum Alpha-feto protein (AFP) expression level is higher in group 3 male participants (middle-aged) and group 4 male participants (Older adults) compared to the other groups. Group 3 and group 4 (except group 2) were statistically significant, *p (p= 0.0154) and **p (p= 0.0050) respectively , compared to the healthy controls. We have no data of AFP for group 1 male participants. (C) The blood Alpha-feto protein (AFP) expression level is normal in all groups of female participants compared to the healthy control groups; ns stand for not significant. (D) Statistical significant difference was observed in gender disparity in any age group of patients. There was a statistically significant increase in AFP expression in male individuals compared to female individuals, *p (p= 0.0214), using a two-tailed unpaired student's t-test. Data represent mean±SEM.

5. Conclusions

Finding the presence of cancer biomarkers in the population lowers mortality and morbidity while also assisting the doctor to establish an approach to treatment that can protect lives. Greater ages were associated with greater blood PSA, CA-125, CEA, and AFP levels. Based on the results of our present exploratory investigation, in older adults, blood PSA (prostate-specific antigen) levels were higher. Unfortunately, CA-125 expression was prevalent among females beyond the age of 36 in

this small Bangladeshi population. It was observed an increased expression of the carcinoembryonic antigen (CEA) among the male and female individuals who were older adults. Interestingly, males had higher expression levels of alpha-feto protein (AFP) than females. It is essential to investigate similar cross-sectional studies in other Bangladeshi regions, particularly to observe the actual PSA, CA-125, CEA, and AFP incidents.

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