Usefulness of Our Newly Designed Supplements for Reducing the High Risk of Pancreatic Cancer

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Usefulness of Our Newly Designed Supplements for Reducing the High Risk of Pancreatic Cancer

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Abstract

Early detection of pancreatic cancer became possible by analyzing micro RNA (miRNA) from peripheral blood. We report our new preventive intervention method to reduce the risk of pancreatic cancer by using our original supplements for patients with high risk of pancreas-bearing cancer.

Purpose: The risk determination of pancreatic cancer by using miRNA from blood was examined, and it was investigated whether it was effective to decrease the high risk of pancreatic cancer by using supplements that we newly devised.

Materials and Methods: For 14 adults who are not sick, the miRNA was extracted from the blood. They consisted of 7 men and 7 females, aged between 64 and 78 years old, averaging 67.6 years old. The encompassing analysis was performed using the next generation sequencer, and the risk of pancreatic cancer was calculated as Expression Score (ES). After three months of our supplements (beta-glucan and Methyl-resveratrol) were subjected to take, the risk determination was carried out repeatedly by analyzing miRNA from blood samples. Changes in tumor-bearing risk treated by our tumor risk-reduction supplements were evaluated by ES.

Results: By the proactive intervention method of the tumor-bearing reduction supplements, the expression scores, in 11 cases (79%) in 14 cases, were reduced significantly (Wilcoxon test; $p<0.0052$) after taking supplements. Conclusion for those who have a high risk of pancreatic cancer, we found it possible to reduce ES perfectly by means of our newly designed supplements. This shows an effective practice of preemptive medicine.

Keywords: High risk; Methyl-resveratrol; microRNA; Newly designed supplements; Next generation sequencer; Pancreatic cancer; Preemptive medicine; Prevention; Beta-glucan,

Abbreviations: miRNA: microRNA; CT: Computed Tomography; MRI: Magnetic Resonance Image; PET: Positron Emission Tomography; ES: Expression Score

Introduction

Diagnosis of pancreatic cancer in its early stage is difficult. Since pancreatic cancer usually found in Stage IV, there is no effective therapy for it [1]. The 5-year survival rate among patients with pancreatic cancer is 9%, which is the lowest among all cancers. Therefore, early detection of pancreatic cancer is desirable. Early detection of pancreatic cancer has become possible by analyzing microRNA (miRNA) from the peripheral blood [2]. Analysis of miRNA allows for detection of pancreatic cancer foci as small as 0.1 mm in diameter. The sensitivity of this test is more than 96% [3]. Because of this sensitivity and specificity of the test, the location of the tumor may not be indicated by conventional radiologic modalities such as CT, MRI, and PET-CT. Thus pancreatic cancer is often not found by imaging, it is a problem that the technical innovation of the diagnostic technology using miRNA analysis does not relate to treatment at once.

In order to make effective use of the early diagnosis method of high sensitivity, we found a method for reducing the risk of cancer bearing. We examined the ability to determine the risk of pancreatic cancer using miRNA from blood. At the same time, we also investigated whether our newly developed supplements can efficiently decrease the risk of pancreatic cancer. We will report here our new preventive intervention method to reduce the risk of pancreatic cancer using our original supplements for patients with a high risk of pancreatic cancer.

Materials and Methods

The approval to perform this study was obtained from the ethical committee of Southern Tohoku General Hospital (acceptance No.; 360). Documented informed consent was obtained from each subject. Blood samples were obtained from 14 healthy adults for miRNA extraction. The patients consisted of seven men and seven women ranging in age from 64 to 78 years (average, 67.6 years). RNA extraction was performed using standard protocol of commercial miRNeasy 96 Kit (Qiagen, Netherland). Total RNA concentration was evaluated by Nanofrop2000 spectrometer (Thermo Scientific, USA). Quality assessment of total RNA samples was performed using Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Small RNA libraries were prepared using TruSeq Small RNA Sample Preparation (Illumina) according to the manufacture's protocol with 1 ug RNA input per sample followed by RNA 3' adapter ligation, RNA 5' adapter ligation, cDNA synthesis, PCR amplification using unique barcode sequences for each sample and gel size-selection of small RNA library. The yield of sequencing libraries was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies). Multiplexed libraries were sequenced on Ion Gene Studio S5 next-generation sequencing platform (Thermo Fisher Scientific, Japan), and the risk of pancreatic cancer was calculated as the expression score (ES). The presence of tumors was evaluated by radiographic images such as CT (160 channels, GE, USA), 3T -MRI (Signa, GE, Japan) and PET-CT (GE, USA). After confirming the absence of visible tumor, the patients took our newly designed supplements orally for 3months. The supplements consisted of beta-glucan (Meshima^{R}, 2 g/day) and methyl-resveratrol (pterostilbene; 2 caps/day).

Risk determination was then carried out repeatedly by analyzing miRNA from the patients' blood samples. Changes in the tumor-bearing risk after treatment with our tumor risk-reduction supplements were evaluated by the ES as calculated by next generation sequencing, just before and after the intervention and followed up 8 months after treatment, then the results were examined by the Wilcoxon test.

Results

The average ES before and after the intervention was -718 and -965, respectively. After the proactive intervention of our tumor risk-reduction supplements, the ES significantly declared in 11 (Wilcoxon test, $p < 0.0052$) of 14 patients (79%) (Figure1). Furthermore, the 14 patients were divided into Group A (n = 8), who shown a higher ES (mean; -564) than the average ES (-900) of healthy persons, and Group B (n = 4), who shown a lower ES (mean; -1007) than the average ES of healthy persons. In Group A, all eight patients (100%) showed a significant reduction of the ES ($p < 0.009$) (Figure 2). In Group B, however, no significant reduction of the ES was obtained ($p = 0.465$) (Figure 3). Supplement for 3 months in group A, without adding further intervention, and 8 months after completion of supplementation, ES were measured in three patients and found it remained unchanged.

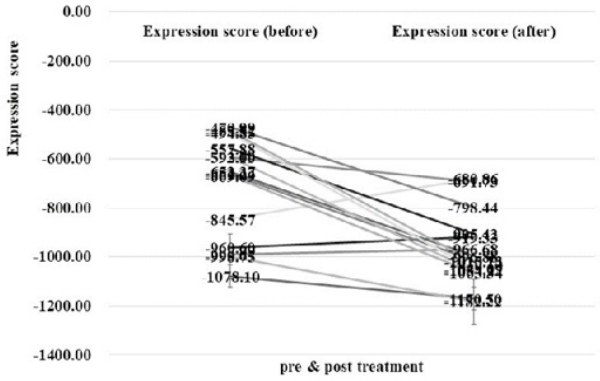

Figure 1: Change in risk before and after intervention in all cases.

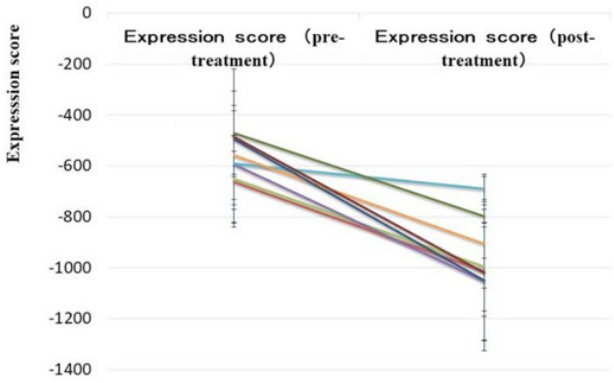

Figure 2: Changes of high risk before and after intervention.

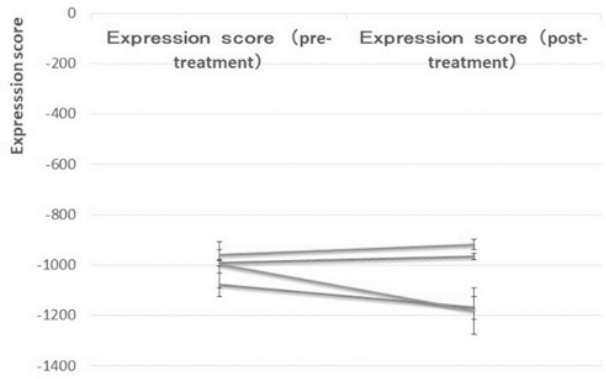

Figure 3: Change in risk among low-risk individuals before and after intervention.

Discussion

Pancreatic cancer remains one of the most lethal malignant neoplasms that caused 432,242 new deaths in 2018 (GLOBOCAN 2018 estimates). Globally, 458,918 new cases of pancreatic cancer have been reported in 2018, and 355,317 new cases are estimated to occur until 2040. Despite advancements in the detection and management of pancreatic cancer, the 5-year survival rate still stands at 9% only [1].

Worldwide incidence and mortality rate of pancreatic cancer in both males and females increases with age, and almost 90% of all deaths occur after the age of 55 years [4]. Pancreatic cancer is very difficult to detect and is often very advanced at the time of detection, making it a deadly and frightening cancer. That's why finding pancreatic cancer as soon as possible is the most important point. Tumor markers used to diagnose pancreatic cancer include CA 19 -9, CEA, Dupan -2, and Span -1. Some tumor markers are more likely to appear in certain organ cancers, but many are made in more than one organ. It can also be elevated in diseases other than cancer (false positive), so high tumor markers do not make a cancer diagnosis [5]. Also, tumor markers are often not elevated in early stage cancers and cannot be used for early diagnosis of pancreatic cancer. Abdominal ultrasonography (so-called abdominal echo)" is a standard and most sensitive imaging examination that may detect pancreatic cancer [6]. Pancreatic cancer must be detected early, when the tumor is 2 centimeters or smaller, but data show that abdominal ultrasound has only a 0.003% chance of detecting pancreatic cancer early. That means it's very difficult to detect pancreatic cancer early in a standard physical examination. Therefore, there are currently no definite guidelines for effective screening for pancreatic cancer.

The miRNA diagnosis used in this study is epoch-making because it has high specificity and sensitivity and can detect early lesions as small as 0.1 mm3. However, diagnosis of pancreatic cancer with such sensitivity is difficult because the neuroradiological system and sensitivity do not catch up, and there is no other way than to closely follow patients. If the ES of the miRNA diagnosis is high, even if it is not visible in the image, the risk can be reduced by the supplement we devised, and if the lifestyle is not disturbed, the reduction in risk lasts more than 8 months. Meshimas are mainly composed of beta-glucan obtained from mushroom hyphae. Beta-glucan is known to activate natural killer T cells, T cells, B cells, and macrophages several times [7]. Pterostilbene also increases the activity of inhibitory miRNAs and exhibits anticancer effects [8].

Meshima^{R}(1 pack/day) and pterostilbene (2 tablets/day) were taken after meals and continued for 3 months. After taking the drug for 3 months, a blood sample is taken, and risk is determined by miRNA. Based on scientific evidence it is suggested that quitting smoking, drinking moderately, eating a balanced diet, physical activity, proper weight, and infection prevention are effective in preventing cancer. Those items are [1] related to daily life habits. By practicing the five health habits, we can lower the chance of getting cancer by our own efforts.

In conclusion, the paradigm of preventive medicine in the future is to use miRNA diagnostics to identify disease risk and, if there is no visible cancer, to take our cancer-reducing supplement for 3 months. After confirming that the cancer risk has decreased, it may be important to observe the five lifestyle behaviors such as quitting smoking, drinking moderately, eating a balanced diet, physical activity, and keep proper weight, and to protect your body so that the cancer risk does not rise again. This is what preemptive medicine should be and is a goal.

Conclusion

For patients at high risk of pancreatic cancer (e. g., those with a high ES), we found it possible to efficiently reduce the ES by means of our newly designed supplies. The risk reduction effect lasted at least eight months. This treatment represents effective practice of preemptive medicine.

Conflict of Interest: We have no conflict of interest.

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CASE REPORT

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Usefulness of Our Newly Designed Supplements (Deriskool A® & B®) for Reducing the High Risk of Eleven Kinds of Solid Cancer

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Abstract

Introduction: An analysis covering small molecule RNA in blood samples was developed (MIA test-platinu ; Miltel®= platina test) to further increase diagnostic accuracy [1,2,3]. Since the comprehensive analysis of small molecule RNA of 11 solid cancers enabled early detection of these solid cancers, we examined whether our developed supplements (Deriskool A®, and B®) significantly reduced the risk of cancer for these 11 solid cancers.

Material and Methods: The subjects were 99 pre-diseased adults. More than 10 million species of small molecule RNA containing miRNA in the blood was extracted and comprehensive analysis was performed with a next-generation sequencer [2,3]. In order to detect various solid cancer patients at an early stage, 11 solid cancers were studied with a responsible cancer risk using the algorithm of biomarkers specific to cancer patients. By diagnostic imaging, if the tumor was not visualized, the cancer carrier reducing supplement of Deriskool A® and Deriskool B® was taken for 3 months. Qualitative changes in the information of small molecule RNAs of the subject and changes in the base sequence were quantified as test values by adapting the risk to the optimal algorithm for each disease. The test value of the carrier risk of each solid cancer was measured by the platina test, before and after supplement intervention was done.

Results: By taking our newly designed supplements, the risk of cancer carrier was significantly reduced in all 11 solid cancers before and after taking them ($p<0.05$, Wilcoxon test).

Conclusion: From the risk assessment of the cancer carrier risk of analysis by the next generation sequencer of comprehensive small molecule RNA in the blood, therapeutic intervention was done with a supplement developed by our hospital, and in all 11 solid cancers, it was possible to significantly improve the cancer carrier risk. This is an effective practice of pre-emptive medicine.

Keywords: High Risk; Methyl-Resveratrol; microRNA; Newly Designed Supplements; Next Generation Sequencer; Solid Cancer; Pre-Emptive Medicine; Prevention; Beta-Glucan; Small RNAs

Abbreviations: MIA Test Premium=Premium Test; MIA Test Platinum=Platina Test

Introduction

Primary prevention through lifestyle and environmental interventions remains the main way to reduce the burden of cancers [4]. Improvements in lifestyle behaviour's to reduce cancer risks include a healthy diet, calorie restriction, and regular physical activity [5,6]. Changes in the metabolism of nutrients such as glucose, amino acid, and fatty acid are associated with cancer risk. Luckily, this can be controlled with lifestyle modification [7]. In addition to lifestyle and environmental interventions, our pre-emptive treatment using newly designed supplement represented effective practice of pancreatic cancer prevention [1]. The supplement we developed consists of beta-glucan; Deriskool A, which activates all immune system cells [6], and Methyl resveratrol; Deriskool B, which activates the Sirtuin gene and also activates cancer-suppressing microRNAs [9]. Taking these supplements for 3 months significantly reduced the risk of pancreatic cancer.

The development of platina test has enabled early cancer detection with high accuracy by small molecule RNA analysis [2] for solid cancers in addition to pancreatic cancer. Therefore, we examined whether our developed cancer risk reduction supplement was effective for newly enabled to examine solid cancer. We report here and emphasize that primary prevention using our newly designed supplements is particularly effective way to prevent for a variety of solid cancer.

Materials and Methods

The approval to perform this study was obtained from the ethical committee of Southern Tohoku General Hospital (acceptance No.; 360). Documented informed consent was obtained from each subject. Blood samples were obtained from 99 healthy adults for small RNA and miRNA extraction. The ratio of males to female subjects who tested for 11 types of cancer risk was as shown in Table 1.

	Male	Female	Total NO.	Mean age(Y)
pancreas	**9**	**5**	**14**	**64.4**
lung	**11**	**8**	**19**	**64**
stomach	**6**	**4**	**10**	**63.8**
liver	**5**	**6**	**11**	**65.4**
esophagus	**4**	**3**	**7**	**64.1**
breast	**0**	**5**	**5**	**62**
colon	**4**	**2**	**6**	**63.5**
Thyroid	**4**	**3**	**7**	**64.6**
gall bladder	**7**	**2**	**9**	**65.6**
kidney	**4**	**2**	**6**	**69**
ovary	**0**	**5**	**5**	**64.4**

Table 1: Composition of Male to Female Subjects

The presence of tumors was evaluated by radiographic images such as CT (320 channels, GE, USA), 3T -MRI (Signa, GE, Japan). After confirming the absence of visible tumor, the patients took our newly designed supplements orally for 3months. The supplements consisted of beta-glucan [8] (Deriskool-A®, 2 g/day) and methyl-resveratrol [9] (Deriskool-B®, 2caps/day). Risk determination was then carried out repeatedly by analyzing small molecule RNA from the patients' blood samples.

Because small molecule RNA has the function of controlling the protein's being made in the cell, the pattern changes early even in the early stage when the state of the function of the cell and the organ changes by the sickness etc. platina test targets small molecule RNA, which changes patterns from the early stages of disease, and considers the presence of early-stage cancer cells that are

difficult to find in images as risks. In order to detect various solid cancer patients at an early stage, 11 solid cancers (i.e., pancreatic cancer, lung cancer, gastric cancer, liver cancer, esophageal cancer, breast cancer, colorectal cancer, thyroid cancer, gallbladder cancer, kidney cancer, ovarian cancer) with a responsible cancer risk using the algorithm of biomarkers specific to cancer patients obtained from the results of next-generation sequencers) (commissioned by Miltel®). [2,3].

RNA sequencing for miR Test

Total RNA was isolated from 200 ml serum using a miRNeasy mini kit (Qiagen) according to the manufacturer's protocol. A cDNA library for small RNA sequencing was prepared by an Ion Total RNA-Seq Kit v2 ((Thermo Fisher Scientific). The size and concentration of base pairs of the cDNA library were measured with an Agilent 2100 Bioanalyzer (Agilent Technologies). The Synthesized templates were sequenced on an Ion S5–XL sequencer (Thermo Fisher Scientific) using an Ion 540-chip.

Data Analysis

The data quality was checked, and analyzed using a CLC genomics work bench 7(CLC bio). Small RNAs expression levels were estimated using RPM values. The normalized reads were annotated to miRBase version 21 with high priority. Remaining reads were annotated to GtRNAdb and GRCh38.p12 as references.

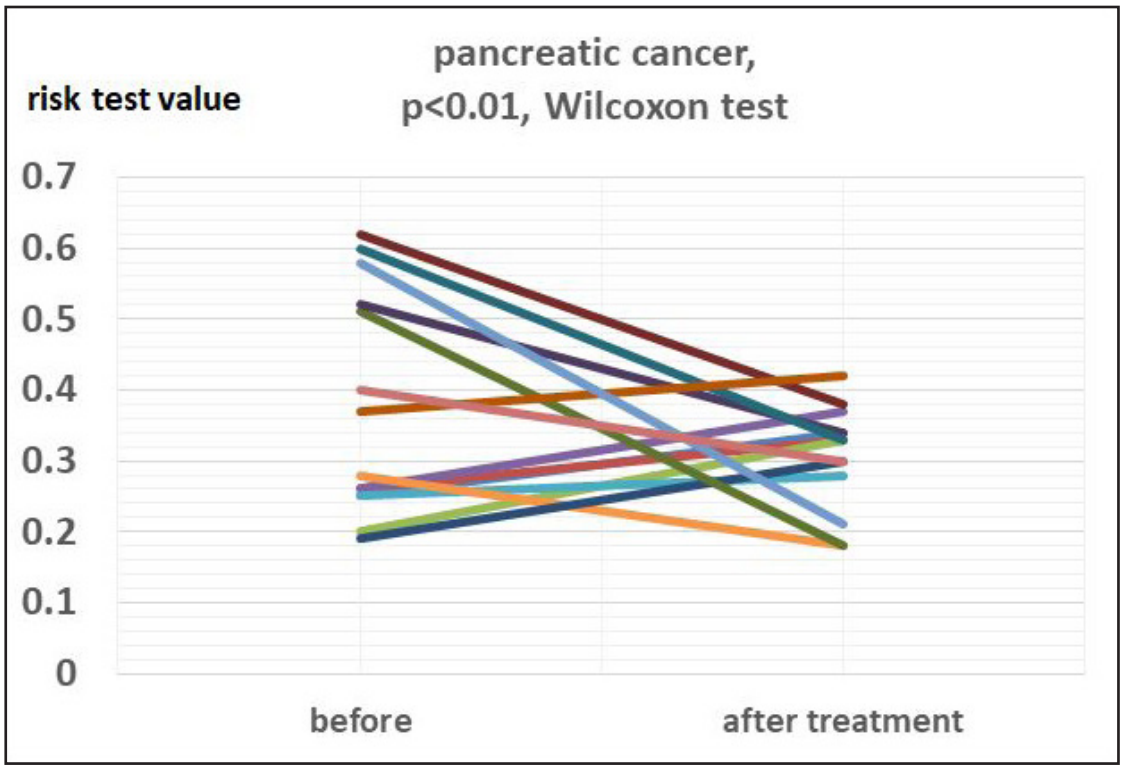
The biomarkers for miR Test were identified by analyzing normalized read numbers of small RNAs between healthy control and each disease group using the Student t-test. A miR Test index was calculated by assigning the read number of some candidates of small RNAs using previously reported method. [2,3] (details of candidate small RNAs of miR Test are not disclosed). All data were statistically analyzed using JMP1 14 (SAS Institute Inc.).

The risk test value distribution was set so that the risk values fluctuate between 0.00 and 1.00. Based on the threshold (test value = 0.5), which accurately separates healthy and disease groups, the test value of less than 0.5 is a low-risk group, and the test value of 0.5 or more is a high-risk area. The low-risk group is divided into 2 minutes based on the intermediate value = 0.25 of healthy subjects, and the high-risk region is divided into 2 minutes based on the intermediate value = 0.75 of the disease group. In this study, it was recommended to take supplements as disease groups and high-risk groups with a risk value exceeding 0.35. Changes in the tumor-bearing risk after treatment with our tumor risk reduction supplements were evaluated by the risk test value as calculated by next generation sequencing, just before and after the intervention then the results were examined by the Wilcoxon test. Significance threshold was set at $p<0.05$.

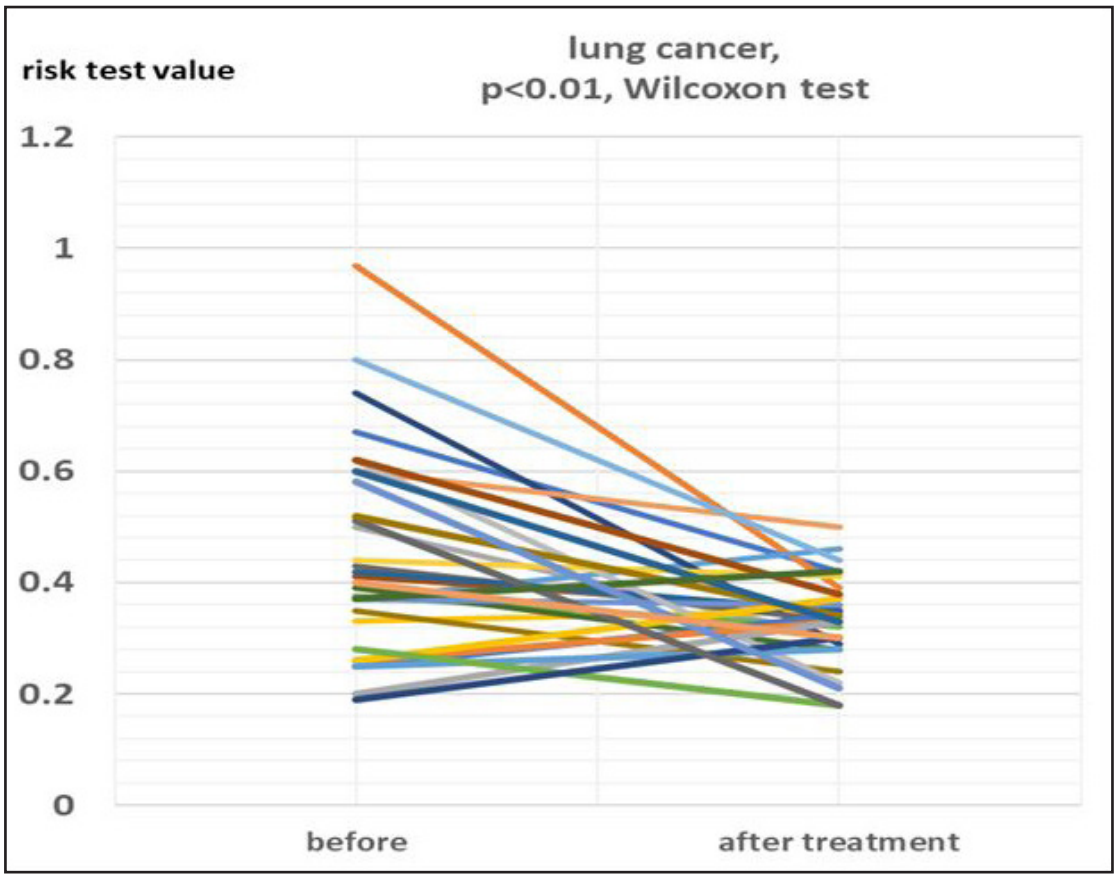
Results

A total of 99 subjects were composed of 54 males and 45 women. The average age of the subjects was 64.6 years old (Table 1). Table 1 shows the average age of the subjects in each solid cancer.

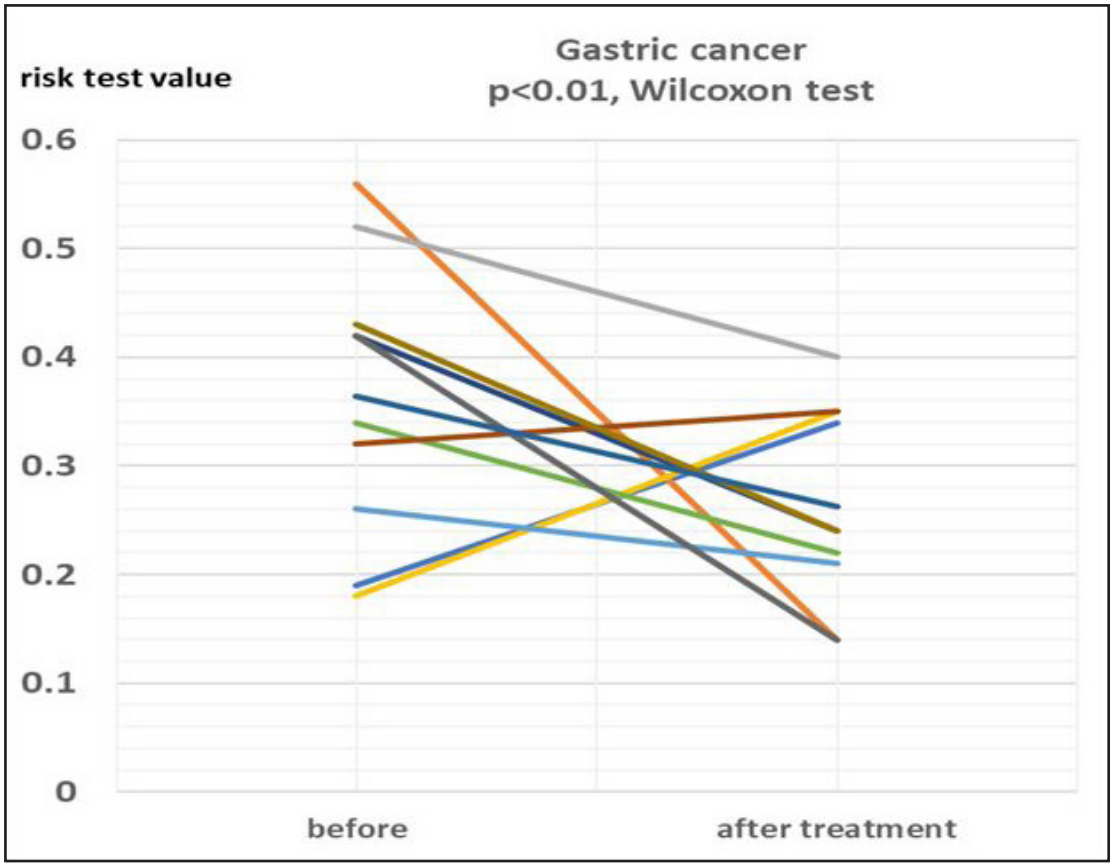
By taking Deriskool A® and Deriskool B® for 3 months, the risk of cancer carrier was significantly reduced in all 11 solid cancers before and after taking. (Figure 1: a~k).



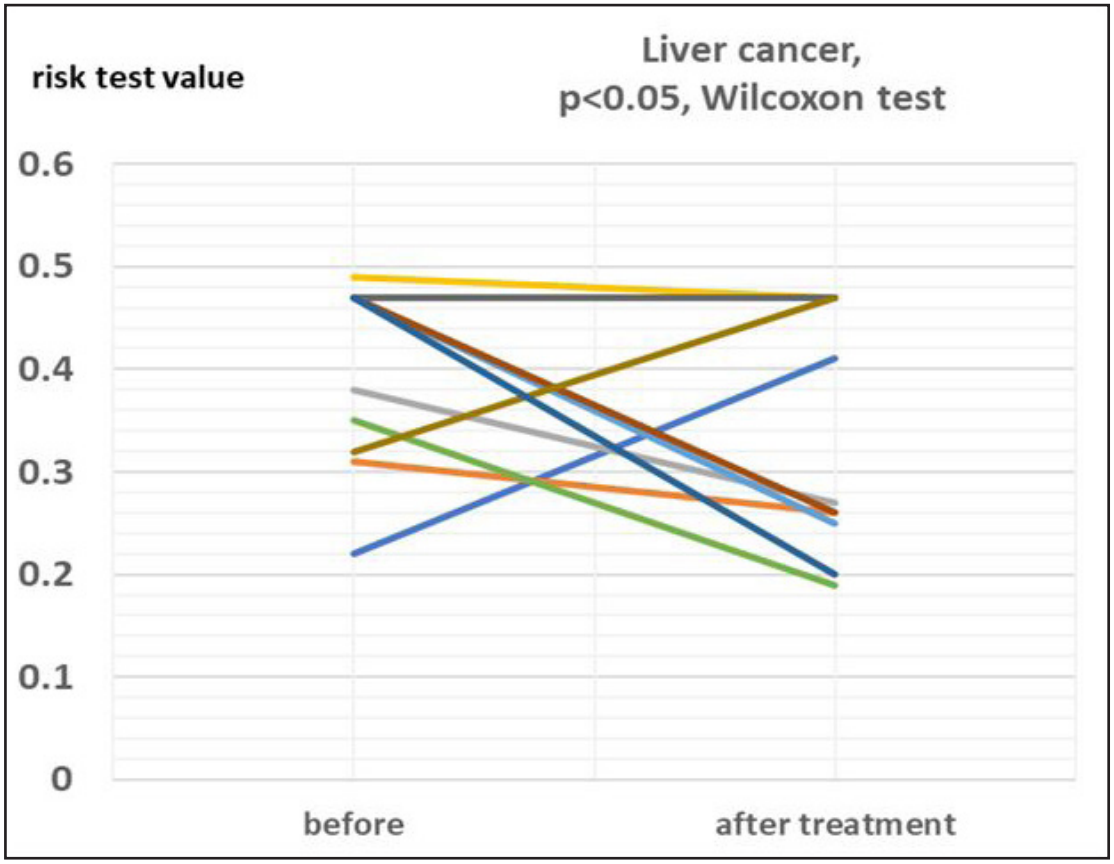
a) Pancreatic cancer



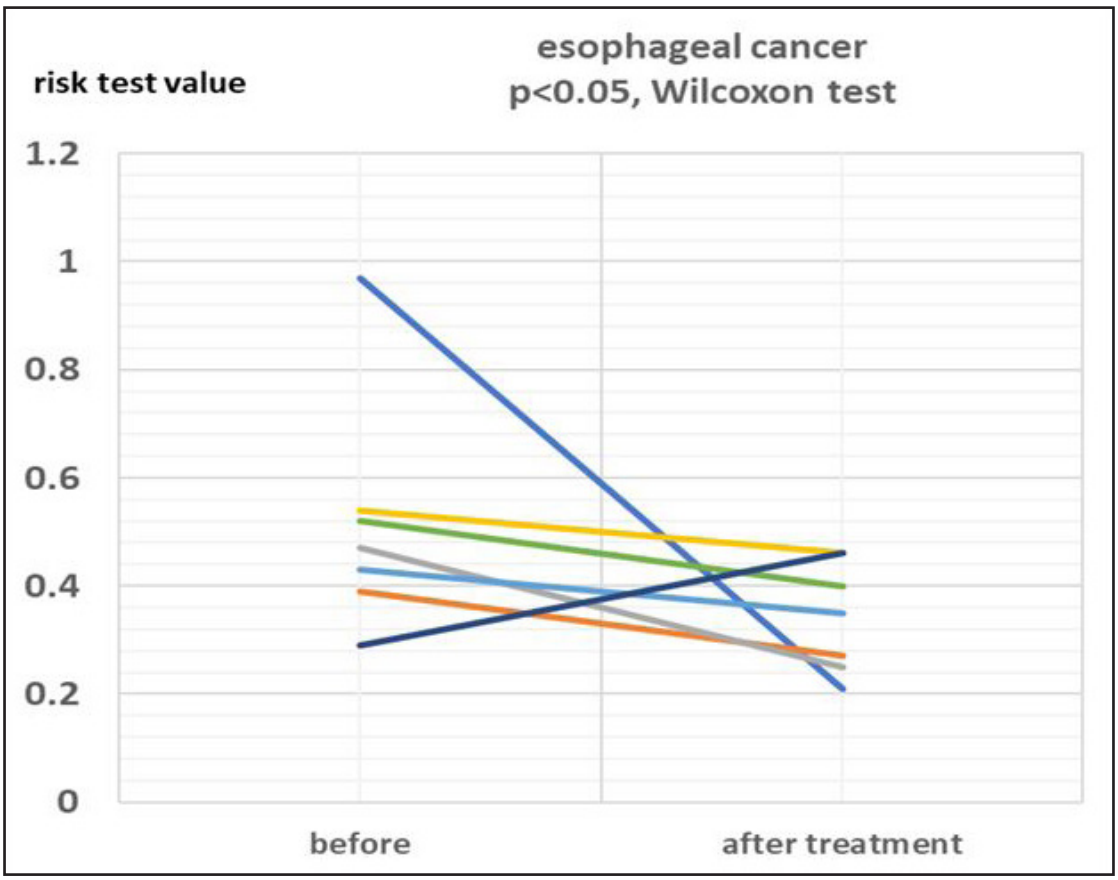
b) Lung Cancer



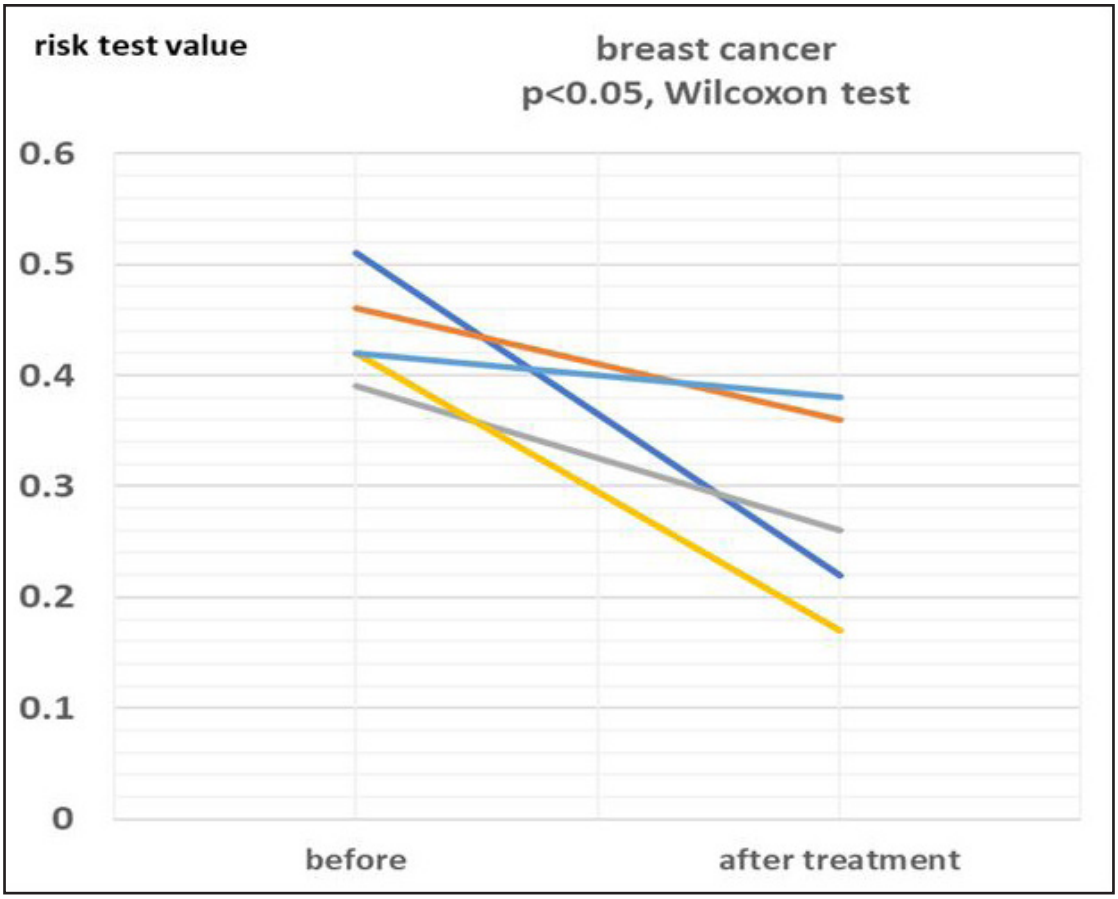
c) gastric cancer



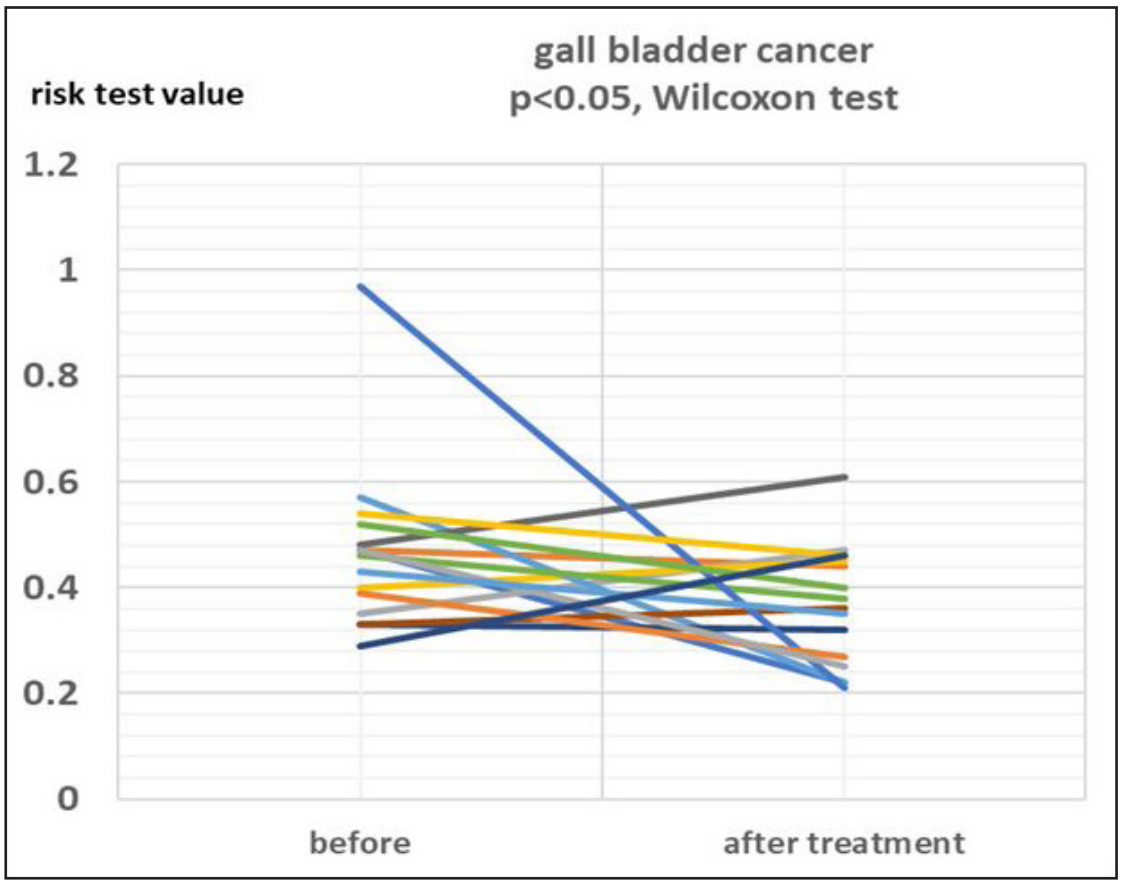
d) Liver Cancer



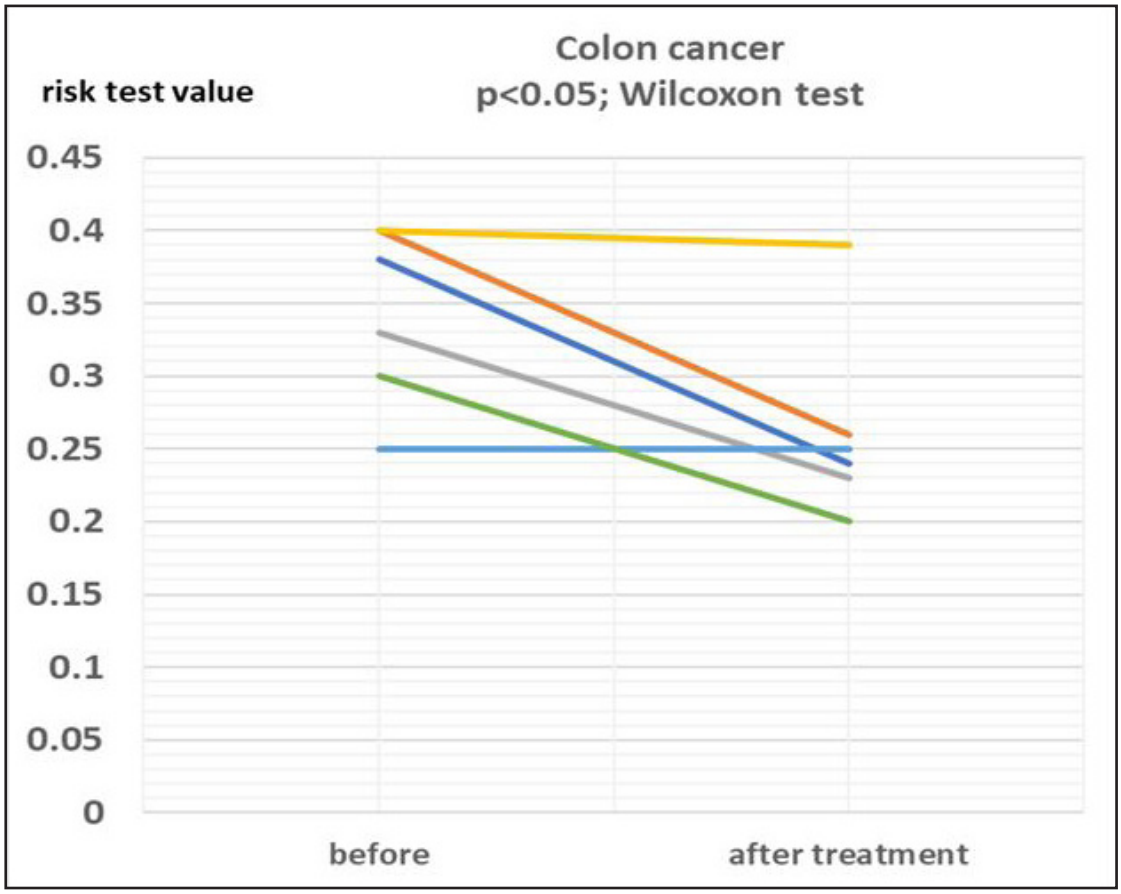
e) esophageal cancer



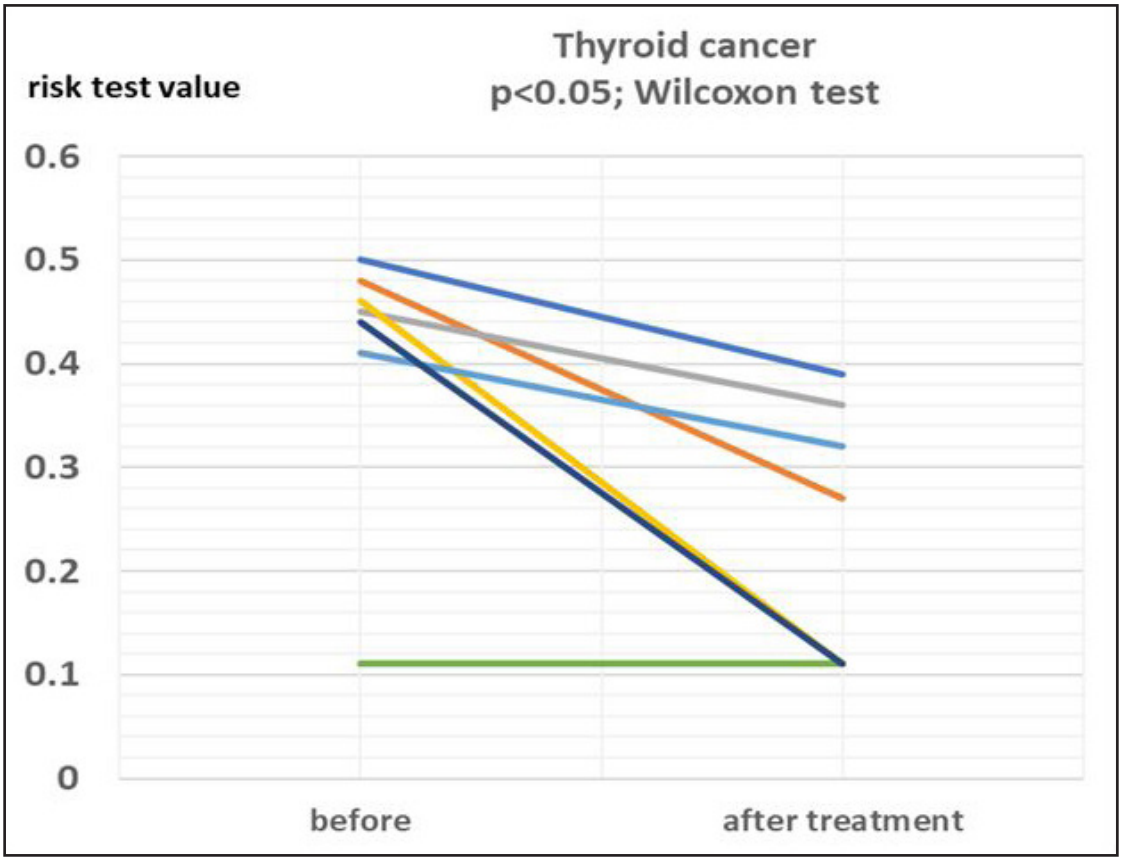
f) Breast cancer



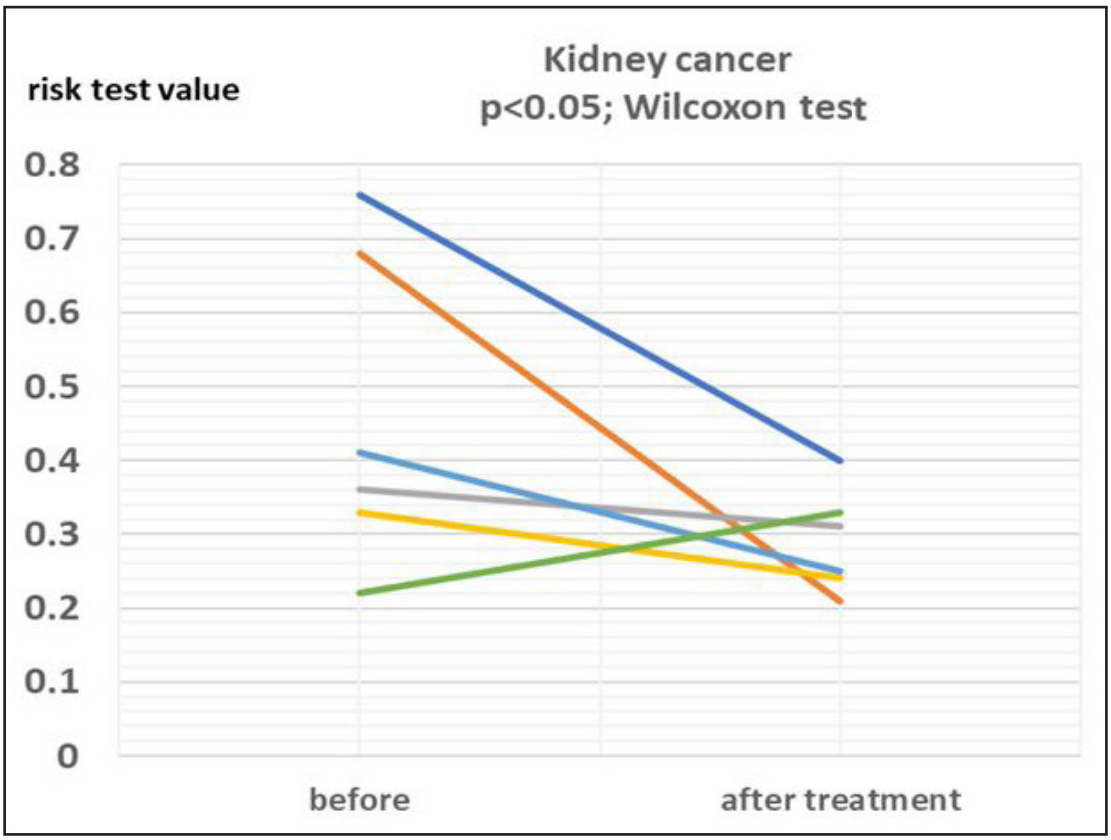
g) Gall bladder cancer



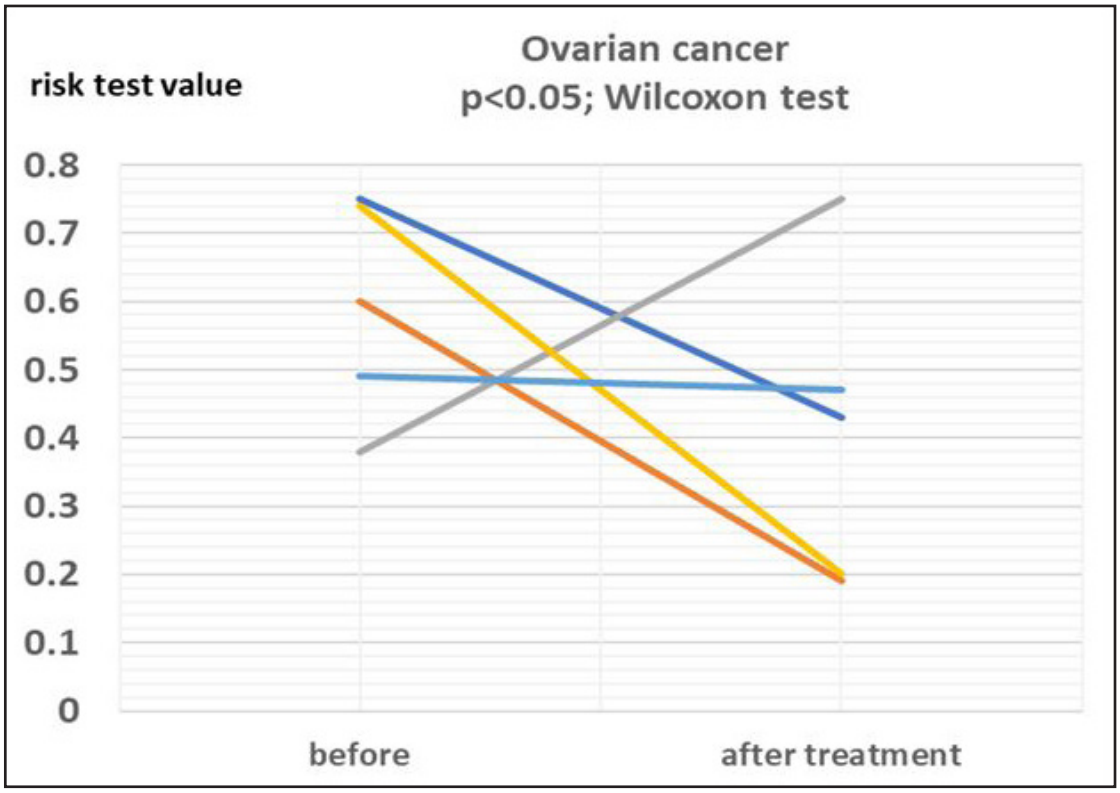
h) Colon cancer



i) Thyroid cancer



j) Kidney cancer



k) Ovarian cancer

Figure 1: Change in risk test value before and after intervention in all cases

Discussion

Cancer is the top leading cause of death in Japan. Despite the advancement in screening, early diagnosis, and development in treatment technology in last several decades, cancer incidence overall is far from being controlled. To avoid important causes of cancer such as smoking, alcohol use, overweight and obesity, irregular physical exercise, and low fruit and vegetable intake, is important [4]. It is also essential to take preventive measures other than to correct these lifestyles, that is, to catch the risk of cancer at an early stage and to picking buds.

In recent years, it has been reported that microRNAs are useful for early diagnosis and treatment of cancer [10,11]. Earlier, we used premium test to determine the risk of pancreatic cancer and find that our developed supplement (Deriskool A® and B®) significantly lowers the risk of pancreatic cancer. The premium test was only evaluated for the expression level of microRNAs (several types) specific for pancreatic cancer. Then, the result was compared with that of the pre-diseased person, and the cancer carrier risk was calculated.

Platina test, which evolved the *premium test*, has made it possible to determine the risk of responsible cancer for 14 solid cancers. This comprehensively performs next-generation sequencer analysis of all data of small molecule RNA in the blood (more than 10,000 types) and obtains sequence information of all small molecule RNAs present in the blood. miRNA, isomiR, t-RNA fragments, ncRNA (other non-coding RNA) using all vast amounts of data to match a genetic database is analyzed. Since the expression level and sequence information are also evaluated, *platina test* is a test with higher accuracy and improved disease specificity. Both sequence information and quantity are analyzed based on the obtained information, and the risk is scored by comparing it with various disease-specific databases [2,3]. In this paper, the cancer carrier risk was examined for 11 kinds of solid cancers in which the number of samples which can withstand the statistical analysis was obtained using *platina test*.

In precision diagnostic imaging (3Tesla-MRI, 64-channel helical CT, and gastrointestinal endoscope), we conducted treatment interventions with our supplements when cancer could not be confirmed. The diagnostic method using small RNA used in this study is epoch-making because it has high specificity and sensitivity [2,3] and can detect early lesions as small as 0.1 mm3. Therefore, it is considered that there is a high possibility that minute lesions that are not understood by diagnostic imaging are found.

The intention of our supplements was to reduce or eliminate the risk of cancer by increasing immunity to cancer [8] and supplementing the progression to cancer triggered by microRNA instability with cancer-suppressive microRNAs [9].

Deriskool A® (Meshimas) are mainly composed of beta-glucan obtained from mushroom hyphae. Beta-glucan is known to activate natural killer T cells, T cells, B cells, and macrophages several times [8]. *Deriskool B*® (Pterostilbene) also increases the activity of inhibitory miRNAs and exhibits anticancer effects [9]. *Deriskool A*® (9 tablets/day) and *Deriskool B*® (2 tablets/day) were taken after meal and continued for three months.

As a result all 11 cancers, after taking our developed supplements, significant ($p < 0.05$, Wilcoxon test) improvement of the cancer risk was obtained.

In conclusion, the paradigm of preventive medicine in the future is to use miRNA diagnostics to identify disease risk and, if there is no visible cancer, to take our cancer-reducing supplement for 3 months. After confirming that the cancer risk has decreased, it may be important to observe the five lifestyle behaviors such as quitting smoking, drinking moderately, eating a balanced diet, physical activity, and keep proper weight, and to protect your body so that the cancer risk does not rise again. This is what preemptive medicine should be and is a goal.

Conclusion

For patients at high risk of a variety of solid cancer, we found it possible to efficiently reduce the risk value of cancer by means of our newly designed supplies. Early evaluation of cancer risk is important and spinning its buds are powerful measures to prevent cancer. The supplements we developed proved to be one of the most powerful means to prevent cancer.

Conflict of Interest

We have no conflict of interest.

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