Investigation of The DNA Damage of White Blood Cell in Cancer Patients Under Chemotherapy and Normal People

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Based on the results obtained in this experiment, this report suggests that the alkaline comet assay is a highly sensitive exposure biomarker that plays an active role in the rapid and easy detection of primary DNA damage in leukocytes of specific patients can be concluded to be one of play with cancer. However, a few steps should be used to ensure the best approach to the subject is achieved. Based on the information presented in this review, using small white whole blood successes is the best approach as it is a simple and non-traumatic method of sampling. Makes the assay suitable for conventional applications in human biomonitoring. In conjunction with standard cytogenetic endpoints, we take responsibility for providing a clinically robust diagnostic approach for the routine detection of significant lesions occurring in leukocytes after delivery of antineoplastic agents.

Introduction

1.1 Background

DNA damage is one of the aspects that can be considered a biomarker for toxicity and response to chemotherapy. However, based on the previous research that has been done n the subject matter, it has remained noted that it is not well known if the concept is highly associated with chemotherapy-induced genotoxicity and malnutrition. Therefore, based on that concept, it is illustrated that this notion is not well established, thus creating a research gap that has not been evaluated in the past numerous decades. In the survey carried out by Chacón & Costanzo, [1] in the evaluation of triple-negative breast cancer, they asserted that there are various types of cancer, which are associated with hormone and growth factors of an individual and genomic description, which has played a focal point in the illustration of the various subtypes of the cancers that are exploited with diverse people in the clinical significance of view. Consequently, another survey was carried out by Walsh et al. [2] an evaluation the spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. Based on the result that they demonstrated in this evaluation, it was revealed that only 5-10% of breast cancer are only believed to be caused by germline mutation [3].

On the other hand, it is noted that many of the same genetic aberrations present inheritable cancers are present in individuals without genetic pre-dispositions [2]. DNA damage is one of the remarkable aspects of taking a significant evaluation on the subject matter. It has been reviewed as one of the fundamental problems among many people in life. From that point of view, it has been noted that DNA damage is one of the significant causes of cancer among many individuals. Technically, based on the survey done by Chacón & Costanzo [1], it was opined that DNA damage is the change of the basic structure of DNA that is not itself replicated when the DNA is replicated.

1.2 Objective

1. To investigate DNA damage of white blood cells in cancer patients under chemotherapy and ordinary people

1.3 Project outline

This study is organized into five main sections. I discuss the background of the study in beginning, study problem, and objective of the study. Part II offers the literature review of the DNA damage of white blood cells in cancer patients under chemotherapy and normal people. The third part will present the research method, sampling, data collection procedure, and analysis. Part IV shows the results of the statistical analysis. Part V presents a discussion of the study findings.

Literature Review

Previous research shows an increasing interest in the approach that can be used to detect the damage produced after cancer chemotherapy. According to Rigaud et al. [4], peripheral blood lymphocytes are highly employed given the target cells' determination of the correlation between exposure of cancer patients to the antineoplastic drug or alteration and radiation of DNA repair efficiency. The main reason why they are highly used is that, following the Vivo exposure to antineoplastic drugs, there is the production of the various liaison of DNA that are induced. Among them, the bae damages include single-strand breaks (SSB), double-strand breaks (dub), apurinic or apyrimidinic (AP) sites, DNA-protein crosslinks, DNA-DNA interstrand crosslinks, and DNA intrastrand crosslinks [5].

However, the DNA damage that the antineoplastic agent induces is influenced by the cell cycle stage, specifically during the time of treatment. In that view, most of the liaisons happen during the DNA synthesis (S) phase. The main reason that is behind this conclusion is due to misreplication. Therefore, when they are unrepaired, the formed liaisons will result in the rise of the chromatid-type aberrations, especially during the (S) phase, and they thus, temper with the transcription and replications of the DNA, which results in cytotoxic and mutagenic impact [6]. Various approaches can be used to monitor the biological impacts, which most previous scholars have suggested among the methods that should be considered as an internal dosimeter, specifically in detecting the increased genotoxic and sumably risks, including those responsible for the provision of information regarding the damage o patient cell; detection of chromosomal aberrations, sister chromatid exchanges, and micronuclei in peripheral blood lymphocytes. Nevertheless, it has been revealed that most of the method is limited to the value, given that DNA damage procession to the microscope and cell proliferation is the key factors for consideration [7]. According to Collins et al.

[8], it was demonstrated that some modern epidemiological studies used alkaline single-cell microgel electrophoresis or comet assay are among the highly used biomarker for the exposure [9]. They demonstrated that this approach is highly applied since the small florescent microscopic approach can be used to detect the DNA damage and measure the repair kinetic at the level of single-cell [10].

Further, it has stayed evaluated the modification variety of the comet assay has allowed it to detect single-strand DNA breaks, alkali-labile sites, double-strand DNA breaks, incomplete excision repair sites, and interstrand crosslinks. Further, it has been applied since it has aloes managed to offer a possibility of allowing studies of different DNA repair pathways like base excision and nucleotide excision repair. Most importantly, it has also played a focal point in allowing DNA fragmentations associated with death or apoptosis to be evaluated.

Patients and Methodology

This survey employed ten patients (8 females and two males who were non-smokers) along with diverse solid cancers. The diagnosis used among the patient was manufactured at Hong Kong University, where they went through chemotherapy after that. The patient's age was between 37 and 58 years, which presented a central average of 49.2 years. All the patients that were included in this evaluation had never gone any previous radio or chemotherapy. There was a signing of the informed consent before understanding the study. To understand the non-traumatic condition among the patients, all the blood samples were collected with the help of venipuncture. All the process was carried out in line with high ethical standard. All the patients were treated with antineoplastic drugs throughout the experiment based on the five diverse chemotherapy procedures, which was polychemotherapy.

The peripheral sample contained 5 ml of venous blood for the blood sample, which was collected and placed in heparinized vacutainer tubes. The sterile condition was observed during the collection of the blood sample. All the blood samples were collected from the patient one day prior to the day of the chemotherapy from each patient. There is consideration of the response of the peripheral blood white cell to the therapy for the analysis of the blood sample taken immediately after ending the intravenous administration of the antineoplastic drugs in the first therapy round. The next step that followed was coding the blood sample immediately after venipuncture. After that, the blood sample was cooled at + 4 degrees Celsius and taken to the lab. The next step was processing the blood sample with the help of the alkaline comet assay.

3.1 Comet Assay

The comet assay was carried out with the employment of the alkaline condition. All the fully frosted slides gained were covered by 1% of the normal melting point agarose. The gel stayed scrapped from the slide immediately after solidification. After that, it is followed by a gel coating with 0.6% NMP agarose. After that process, a second layer that contained the whole blood sample (4 µl) mixed with 0.5% low melting point (LMP) agarose was placed on the slides. It followed 10 minutes of solidification with 0.5% LMP agarose. After 10 min of solidification on ice, slides were covered with 0.5% LMP agarose. Afterward, the slides were immersed for 1 hour in ice-cold freshly prepared lysis solution (2.5 M NaCl, 100 mM Na2EDTA, ten mM Tris-HCl, and 1% Na-sarcosinate, pH 10) with 1% Triton X-100 and 10% dimethyl sulfoxide added fresh to lyse cells and allow DNA unfolding. After that, placing the gel horizontally in the electrophoresis tank while facing the anode was a procedure. The electrophoresis and denaturation were all conducted at +4 degrees Celsius in dim light. Electrophoresis was carried out for 20 min at 25 V (300 mA). After electrophoresis, the slides were rinsed gently three times with a neutralization buffer (0.4 M Tris- HCl, pH 7.5) to remove excess alkali and detergents. Each slide was stained with ethidium bromide (20 µg/ml) and covered with a coverslip. Slides were stored at +4°C in sealed boxes until analysis.

3.2 Analysis and Comet Assay Capture

In this evaluation, 50 comets per slide were captured randomly in a congruent depth of the gel. All of them were analyzed at ×400 magnification in a fluorescence microscope, which stayed connected via a black and white camera, a computer-based imaging machine. The central comet assay system employed in this survey was Assay II from the United Kingdom, which was employed for the automatic evaluation of the digitized image. For the analysis process that was used in quantifying the DNA damage, there was a need for the application of two diverse parameters, which were tail length and tail moment. The tail length was associated with DNA fragment size and the centra presentation in the micrometers. The calculation was conducted from the center of the cell. On the other hand, the tail moment remained evaluated as the products of the tail and the fraction of DNA in the comet tail.

3.3 Data Analysis

T-test of the dependent sample was compared based on the mean value difference between TL and TM when they were measured in the blood sample just immediately after the chemotherapy and their respective pre-treatment baseline. In that regard, the difference gained from pre and post-test treatment of DNA level damage in the peripheral white blood cell stayed compared with the mean of the t-test for the independent samples.

Results

The result gained in this evaluation was the TL and TM with corresponding minimum and maximum values measured in the white blood cells of the concept patients, which was before and after the chemotherapy. Therefore, based on the above evaluation, it is illustrated that the first result that is presented is the pre-treatment of DNA damage in white blood cells. In lite of the TL, it was noted that it ranged from 13.47 ± 1.34 µm to 16.17 ± 1.18 µm, and all of the lengths were experienced among patients with breast cancer. Therefore, the length measurement was gained from patients who had different types of tumors, such as breast and colon cancer. On the other hand, for the TM, it was found that the mean value of the comet tail measured prior to chemotherapy ranged from 10.81 ± 1.30 to 13.61 ± 1.13 among patients with different types of cancer. Therefore, the logic behind this line of thinking is that white blood cells from cancer patients evaluated before the chemotherapy demonstrated interindividual variation initiallyinitially. At the same time, there was a presentation of DNA damage. After an introduction of the antineoplastic drugs post-treatment, it had noted that all cancer patients showed a significantly increased DNA damage level when it was compared to the pre-treatment values.

It is logical to present that this research found out that the highest DNA damage level was found among patients who received FAP protocol, with a mean value of 21.22 ± 2.07 µm. in the patient, it was noted that the difference between the pre and post-treatment value was found to be 7.39 µm or tail length. Therefore, this result can be used to conclude that there is a strong response to the chemotherapy that was used in this evaluation. On the other hand, the patients used in this experiment showed the lowest mean value of 16.88 ± 2.50 who received FAC protocol. However, there was a minor difference in the value of the pre-and post-treatment, which was 1.29 µm tail length. On the other hand, for the tail moment that was measured among the same patients after chemotherapy, it was found that it ranges from 14.17 ± 2.37 to 18 ± 1.96. therefore, based on this finding, it is logical to argue that the patient happened to show diverse sensitivity towards the DNA damaging impact of the chemotherapy. The highest TM ratio was 1.62, and the lowest was 1.09 for the patient who received FAP and FAC protocol.

In the comparison of the comet assay measures in parameters in white blood cells of cancer

patients that were administrated with the same chemotherapy protocol, it was found that the patient showed interindividual differences concerning both the comet tail parameter that were recorded. Therefore, this indicates that all the patients administrated in the white blood cells, specifically among the mean value o the comet tail, were statistically significant apart from patients who received FAC protocol. Another significant result gained in this survey was the result gained from comparing the comet parameter measures for inpatients who received different chemotherapy. This evaluation revealed that the relative effectiveness of the comet assay alkaline was FAP > 5FU- LV ≥ CMF > ACOP ≥ FAC.

Discussion

In this evaluation, alkaline comet assay was employed as a biomarker, op that it can be used to evaluate the level of the DNA damage of white blood cells among patients and normal people. This research predicted that there is a harmful effect that will be gained from the application of the antineoplastic drugs on DNA damage among cancer patients undergoing chemotherapy than normal people, specifically those using cyclophosphamide and other alkylating agents [11]. This research has shown that there is significant DNA damage in white blood cells in the administration f the antineoplastic in the standard protocol. This research is congruent with the survey done by Hellman et al. [10]. They asserted that the application of antineoplastic drugs is associated with DNA damage, specifically among cancer patients under chemotherapy. However, according to most of the human experiment that has been carried out previously, it has been noted that most of the DNA damage was evaluated in the isolated white blood cell. The study supports this aspect carried out by Kevekordes et al. [12]. They demonstrated that the application of fresh blood tends to positively influence the detection of the effect of DNA while containing 2/3of the short construct. Ideally, based on their evaluation, it was noted that the application of the isolated blood cells is vital in this evaluation, given that it allows detection of the chronic impact of DNA.

In consideration of our evaluation, it is noted that a whole sample was used since it is one of the approaches that are not time-consuming. It remained significant in the analysis of the primary DNA damage that was highly established by antineoplastic drugs, specifically in somatic cells of the cancer target population [13]. On the other hand, if this study had been a longitudinal survey, the allocation of the alkaline comet assay in isolated cells would have been the most appropriate. In that regard, based on the evaluation that was gained from this assessment, it was noted that there was different inter-individual variability in DNA damage baseline in white blood cells, especially from different patients, which is congruent to the survey done by Vaghef et al. [14] and Hartmann et al. [15]. Further, it was noted in this research that the level of DNA damage in cancer patients was higher than in normal people. This study finding is consistent with the experiment carried out by Vaghef et al. [14]. Reasoning along with the baseline point of view, it has been noted that the personal-life styles highly influenced the level of the DNA damage. It is logical to argue that the notion of cancer-prone syndromes is typically associated with the notion of high DNA games among individuals. Further, the above factor has been associated with lower DNA repair induced by genotoxins [15].

Reasoning along with the report that previous scholars have documented, most cancer patients are associated with the notion of illustrating different sensitivity and interindividual variability to respond to an identical scheme of chemotherapy. This research has been noted that it is congruent with the previous researchers' findings since the patient who were included in the survey showed the same result [16]. On the other hand, given that the patient used in this research received different types of procedural treatment, specifically polychemotherapy, it remained very hard to establish the relationship between the dose of specific antineoplastic drugs and the effects measured. The central aspect that explains this notion is that modern chemotherapy has applied the concept that allows the combination of drugs, as opposed to the application of a single drug. The main reason modern therapy uses the combination is that it has the capacity to produce

additive cancer cells while overcoming the probability of emergency of drug-resistance cells among patients. Therefore, most of the target population used in this evaluation were administered with alkylating drugs and a combination of antimetabolites. For this evaluation, it was noted that there was a higher level of DNA damage observed among alkaline comet assay, which was followed with the application of the FAP protocol. The logical explanation behind this finding is that the highest response observed in the therapy was due to the existence of additives.

Further, it is logical to argue that applying each of the drugs tends to have a significant DNA damaging possibility. The experiment supports this study finding carried out by Dabholkar et al. [17], where they documented that the existence of the subsequent exposure in the process of the chemotherapy in the white blood cells of the cancer patients, there is numerous single-strand break that is experienced for the DNA fragment, which is responsible for being measured by comet assay are highly provoked. This research finding is responsible for explaining the aspect that was experienced in this survey.

It remains important to note that most of the patients that were included in this evaluation were highly administered with cyclophosphamide. This agent was responsible for the induction of the diverse DNA base modification. For that reason, the experienced DNA liaison remained repaired with specific enzymes that recognized the alkaline base, which is responsible for producing the base loss. Based on the result that is gained in this experiment, after the provision of the therapy procedure that included cyclophosphamide, the DNA damage level that was observed in white blood cells among all the patients in the experience hoed a significant increment compared to the level that was observed in the pre-treatment [18]. On the other hand, it was noted that reasoning along with the mechanism of cyclophosphamide action, it is logical to argue that the level of the DNA damage in white blood cells from the target population was higher compared to the detected mean of the present procedure. From that point of view, it is logical to argue that cells with DNA crosslinking lesions happened to indicate no increment or a decrement of the DNA mitigation in the applied alkaline comet assay [19]. This result is supported by one of the remarkable previous research projects that focused on treating patients treated with an alkylating agent. The report documented that there was a cause of linear decrement of the tail moment, specifically in the first hour, immediately after treatment [20]. Therefore, another concept that can be used to explain this kind of result is a notion connected with the time of sampling after the therapy session. This is supported by the fact that the relationship between DNA damage onset is associated with exposure time. The two variables are also associated with the mechanism of DNA damage and the measured damage degree level, resulting from the equilibrium between damage infliction and repair period [21]. This study ensured that the blood sample was collected an hour after ending intravenous administration of the used drugs to overcome these issues. Therefore, this aspect played a focal point in ensuring that the comet assay detects only the significant proportion of the real DNA damage induced in the white blood cell by the alkylating agent. To attain the most reliable result, it is important to apply the comet assay proposed by Hartley et al. [20]. Another logical approach that can be applied is the combination of the alkaline comet assay with the sister chromatid exchange [22].

Technically, based on the development of modern therapy, it has played a focal point in ensuring that it increases the survival chance of most cancer patients across the globe. Therefore, the application of chemotherapy has increased its application in most hospitals in increasing the survival chance of cancer patients. It is quite effective in combination with other types of therapy like radiation and surgery. On the other hand, even though they’re some successful treatments have been experienced from the application of this procedure, the possibility that the patient can still be affected, specifically in second malignancy, which might happen many years after the procedures has reduced, still exists. The reason behind this aspect is that there are carcinogenic impacts of the antineoplastic drugs applied in the procedure. They happen to accumulate, which happens to be one of the determinants of the second cancer risks.

Further, previous research is congruent to this finding that the risks created in this evaluation are

correlational to the types of treatment applied. It is high for the patient who is receiving intensive treatment. Specifically, alkylating agents like cyclophosphamide and melphalan are highly connected with second malignant cancer in patients undergoing chemotherapy [23]. This research has played a focal point in the concept that can be used to reduce the second malignant cancer among patients who are undergoing insensitive treatment in chemotherapy. This research has documented a need for consistent biomonitoring of cancer patients, specifically after the end of the chemotherapy procedure.

In conclusion, according to the result gained in this experiment, this report can conclude that alkaline comet assay is one of the remarkable sensitive biomarkers of exposure that is taking a proactive role in allowing rapid and simple detection of the primary DNA damage in white blood cells, specifical patients with cancer. However, some of the procedures need to be used to ensure that the best approach to the subject matter is achieved. According to the information presented in this evaluation, the employment of the small whole white blood success is the best approach, given that it is an easy and non-traumatic way of sample collection. Further, the availability of the white blood cells makes it essay amenable for conventional application in human biomonitoring. In conjunction with standard cytogenetic endpoints, it takes the responsibility of offering powerful diagnostic approaches for the routine detection of the critical lesions produced in white blood cells after providing the antineoplastic from the clinical point of view.

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Statement of Transparency and Principals:

• Author declares no conflict of interest

• Study was approved by Research Ethic Committee of author affiliated Institute.

• Study’s data is available upon a reasonable request.

• All authors have contributed to implementation of this research.

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