Heliyon 7 (2021) e07558

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Determination of O^6-Methylguanine in dried blood spot of breast cancer patients after cyclophosphamide administration☆

Yahdiana Harahap [a,b,*], Athalia Theda Tanujaya [a], Farhan Nurahman [a], Aurelia Maria Vianney [a], Denni Joko Purwanto [c]

[a] Bioavailability/Bioequivalence Laboratory, Faculty of Pharmacy, Universitas Indonesia, Depok 16424, West Java, Indonesia
[b] Faculty of Pharmacy, Republic of Indonesia Defense University, Bogor, 16810, Indonesia
[c] Dharmais Cancer Hospital, West Jakarta, 11420, Jakarta, Indonesia

ARTICLE INFO

Keywords:
Cyclophosphamide
Dried blood spot (DBS)
UPLC-MS/MS
O^6-Methylguanine
Secondary cancer

ABSTRACT

Cyclophosphamide is a nitrogen mustard class of drugs that are often used in cancer chemotherapy. However, the use of Cyclophosphamide in high doses over a long period has been shown to increase the risk of developing secondary cancer. This can be indicated by the formation of mutagenic DNA adducts, such as O^6-Methylguanine. Therefore, this adduct can be used as a biomarker for secondary cancer in patients receiving Cyclophosphamide. Bio sampling was carried out by using the Dried Blood Spot (DBS) method, followed by DNA extraction by using QIAamp DNA mini kit, and acid hydrolysis to obtain O^6-Methylguanine. Analysis of O^6-Methylguanine was performed by using the UPLC-MS/MS instrument with the conditions developed by Vianney, Harahap, & Suryadi (2021). Partial validation was carried out before the analysis. The results obtained from the calibration curve, accuracy, and precision validation test met the FDA requirements. The analysis method was then implemented in 16 breast cancer patients who received the Cyclophosphamide regimen. The O^6-Methylguanine was successfully detected and quantified in all of the samples in the range of 0.55–6.66 ng/mL. It shows that the O^6-Methylguanine accumulation in cancer patients receiving Cyclophosphamide is very likely to occur and the analysis method proposed by Vianney, Harahap, & Suryadi (2021) is potential to be used for Therapeutic Drug Monitoring in this group of patients.

1. Introduction

Breast cancer occurs due to abnormal and uncontrolled cell growth in the breast [1]. The World Health Organization (WHO) notes that every year there are 2.1 million women who suffer from breast cancer and in 2018 it has caused the death of 627,000 women in the world. This makes breast cancer the most common cause of death in women.

One of the breast cancer treatments that can be done is chemotherapy, which is the administration of a single drug or a combination of several drugs. One of the drugs commonly used in chemotherapy is Cyclophosphamide. Cyclophosphamide is a mustard nitrogen-type alkylating agent that works by alkylating or binding to intracellular molecular structures, including nucleic acids. Its activity as a cytotoxic agent occurs due to the cross-linking of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) strands, as well as inhibition of protein synthesis. However, the use of Cyclophosphamide in high doses and for a long period has been shown to increase the risk of secondary cancer [2]. The occurrence of carcinogenesis and secondary cancer can be indicated by the formation of DNA adducts in the blood. This DNA adduct is a compound formed by the addition of large molecules (for example alkyls) to DNA. Alkylation usually occurs in DNA bases, namely at O or N atoms, depending on the alkylating agent used and the type of DNA strand [3]. These compounds can be eliminated from the body through the DNA repair process by enzymes so that the DNA returns to normal conditions. However, when this process is inhibited, miscoding will occur during the DNA replication process, and in the end, it can lead to cancer [4]. One of the DNA adducts that plays an important role in the mutagenesis of Cyclophosphamide is O^6-Methylguanine. The structure of O^6-Methylguanine is shown in Figure 1. Early detection of the presence of O^6-Methylguanine in patients receiving Cyclophosphamide can be used as a way to predict the risk of secondary cancer [5].

Previous researchers have developed a monitoring method for Cyclophosphamide by measuring the AUC value [6] and the antibodies produced against hapten exposed to Cyclophosphamide [7]. Also, some

☆ This study was conducted following the Declaration of Helsinki.

* Corresponding author.
E-mail address: yahdiana@farmasi.ui.ac.id (Y. Harahap).

https://doi.org/10.1016/j.heliyon.2021.e07558
Received 4 May 2021; Received in revised form 27 June 2021; Accepted 9 July 2021

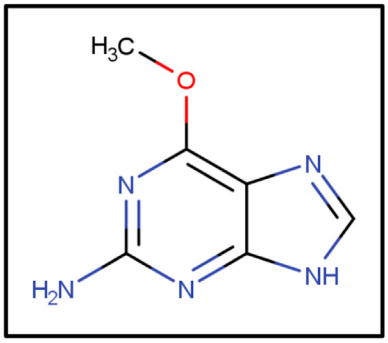

Figure 1. O^6-Methylguanine chemical structure.

researchers have developed an analysis method for the DNA adduct O^6-Methylguanine. The results showed that the integration of the bio sampling dried blood spot (DBS) method, DNA hydrolysis, and analysis using Ultra-High performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) could provide good analysis results for O^6-Methylguanine [8]. However, the therapeutic monitoring of drugs has not been fully implemented in the world of oncology. This is due to many limitations, such as the difficulty in determining the target concentration range and the absence of a simple and comfortable method for patients [9].

2. Materials and methods

This research was reviewed and approved by the Research Ethics Committees of "Dharmais" Cancer Hospital, Jakarta, Indonesia (No.023/KEPK/II/2020).

2.1. Chemical and reagents

Standard materials such as O^6-Methylguanine, N^7-Methylguanine, adenine, and guanine were purchased from Sigma Aldrich (St. Louis, MO, USA). Internal Standard Allopurinol was obtained from Jiangsu Yew Pharm (Yixing, China). Reagents were purchased from Merck Co. Ltd. (Darmstadt, Germany). Ultrapure water was provided by the Sartorius Water Filter System. The sample of human blood was obtained from 16 cancer patients in Dharmais Cancer Hospital (Jakarta, Indonesia) and The Indonesian Red Cross (Jakarta, Indonesia). PerkinElmer 226 papers were purchased from PerkinElmer (Waltham, USA). QIAamp DNA Mini Kits was purchased from QIAGEN.

2.2. Preparation of stock and working standard solution

O^6-Methylguanine and Allopurinol's stock solution were both prepared at 1.0 mg/mL concentration in methanol. The O^6-Methylguanine stock solution was diluted in water containing 0.5% (v/v) formic acid to obtain a working standard. Then, the working standard was further diluted in the blood samples to obtain a series of calibration curve samples in the range of 0.5–20 ng/mL. While the Quality Control (QC) samples were prepared from another stock solution at 1.5 ng/ml (QCL), 10 ng/ml (QCM), and 15 ng/ml (QCH) for O^6-Methylguanine by diluting the working solution in whole blood. Allopurinol working standard was prepared by diluting the stock solution in water containing 0.5% (v/v) formic acid to a concentration of 100 ng/mL.

2.3. UPLC-MS/MS conditions

This research was conducted using methods that have been optimized by Vianney, Harahap, & Suryadi (2021). The analysis of the prepared sample was performed on an ACQUITY™ UPLC system (Waters Corp., Milford, MA, USA) and a Xevo TQD Triple Quadrupole mass spectrometer (Waters Corp., Manchester, UK) with positive electrospray ionization (ESI +) mode. Then, the data were provided in centroid mode by the MassLynx™ NT4.1 software and analyzed by the QuanLynx™ program (Waters Corp., Milford, MA, USA). The analyte was separated on the Acquity® UPLC BEH C18 column (1.7 μm, 100 mm × 2.1 mm, Waters Corp., Milford, MA, USA). The mobile phase was 0.05% formic acid solution and acetonitrile; the injection volume was 10 μL; flow rate 0.1 mL/min; autosampler temperature at 8 °C; gradient elution was used for 6 min and shown in Table 1. The detector on the mass spectrometer is set as follows: capillary voltage of 3.50 kV, nitrogen desolvation temperature at 349 °C with a flow rate of 643 L/h, column temperature of 40 °C, and degasser pressure of 0.69 psi. The cone voltage was 32 V for O^6-Methylguanine, 38 V for N^7-Methylguanine, 40 V for adenine, 35 V for guanine, and 35 V for Allopurinol as IS. The detector was performed in ESI + mode and multiple reaction monitoring (MRM) with ion transition at the m/z value of 165.95 → 149 and 165.95 → 134 for O^6-Methylguanine, m/z 165.95 → 149 and 165.95 → 124 for N^7-Methylguanine, m/z 135.9 → 118.95 for adenine, m/z 151.9 → 134.95 for guanine and m/z 136.9 → 110 for Allopurinol as internal standard.

2.4. Preparation of sample in dried blood spot

The sample of human blood was obtained from 16 cancer patients in Dharmais Cancer Hospital (Jakarta, Indonesia) and The Indonesian Red Cross (Jakarta, Indonesia). To make the calibration standard and quality control samples, 50 μL of whole blood containing O^6-Methylguanine were pipetted onto the PerkinElmer 226 paper and dried at room temperature for 2 h. Then, DBS discs were cut and inserted into a microtube. The internal standard is made to a concentration of 1 μg/mL and 20 μL was added. The sample was extracted using the QIAamp DNA Mini Kit. DNA extraction procedures refer to the QIAamp DNA Mini and Blood Mini Handbook [10] as follows:

1. The Dried Blood Spot sample was placed in a 1.5 ml microcentrifugation tube, then a 180 μL of the ATL buffer was added, and the sample was incubated at 85 °C. Then 20 μL of proteinase K solution was added and the sample was incubated again at 56 °C. After that, 200 μL of the AL buffer was added to the sample and incubated at 70 °C.
2. Samples were added with 200 μL ethanol (96–100%) and the mixture is carefully transferred into the QIAamp mini spin column. QIAamp mini spin column which was used in this extraction consisted of a designed silica layer that can trap the DNA on it when centrifuged.
3. Then, AW1 and AW2 buffer was added to separate protein from DNA, therefore it increased the purity of DNA.
4. Finally, the DNA on the silica layer was eluted using AE buffer and incubated at room temperature. The results of DNA extraction can be stored at -20 °C.

The 50 μL DNA solution was taken and mixed with 50 μL of ultrapure water and 90% formic acid. Then the mixture was heated at 85 °C for 60 min. The mixture must be cooled to room temperature before 15 μL of 1 μg/mL Allopurinol as the internal standard was added into the mixture. The internal standard concentration in the mixture was 100 ng/mL. After the internal standard addition, the mixture was ready to be injected into UPLC-MS/MS.

Table 1. The used gradient elution profile (Vianney, Harahap, & Suryadi, 2021. has been reprocessed).

Time (minute)	Mobile phase A (%)	Mobile phase B (%)
0.00	90	10
1.00	95	5
2.00	95	5
2.10	90	10
6.00	90	10

2.5. Blank sample preparation

The blank prepared with this following method was used for the method development, validation, and analysis. Based on the European Medicines Agency (EMA), the blank used for the bioanalytical method validation should be prepared in the biological matrix which is used in the study. Therefore, the blank used for this study was prepared with blood which was spotted on the Dried Blood Spot paper and through the same process as the sample, but without the analyte and internal standard addition. First, 50 μL of analyte-free whole blood obtained from the blood bank was spotted on the Dried Blood Spot paper and dried for 2 h. Then, the DNA was extracted from the DBS paper and isolated by using the same method as the sample preparation method explained in section 2.4, but the internal standard was not added into the mixture. The blank sample was injected into the UPLC-MS/MS and analyzes with the condition described in section 2.3.

2.6. Method validation

Validation of analytical methods is carried out to ensure that the data obtained is reliable, the methods used are specific, selective, accurate, and precise [11]. There is 3 kind of validation, which are full validation, partial validation, and cross-validation. Since this research used a validated method by previous researchers with minor changes, we only did a partial validation. The parameters that we validated are calibration curve and within-run accuracy & precision. Validation of these analytical methods was assessed according to the Food and Drug Administration (FDA) [11].

2.6.1. Calibration curve

Calibration curves created in the biological matrix to be used in the analysis and the results must be reproducible. The sample of the calibration curve consists of a blank sample (does not contain analytes and internal standards); zero samples (blank containing internal standard); and non-zero samples with a minimum of six concentration variations over the range of quantification, including the lower limit of quantification (LLOQ) concentration at each test. Calibration curve acceptance requirements, namely the concentration of each non-zero sample must meet ±15% of the nominal concentration (theoretical concentration), except for LLOQ which must meet ±20% of the nominal concentration in each validation test. A minimum of 75% or six levels of non-zero samples must meet this requirement at each validation test. If there is data that fails to meet these criteria, then the data is not included [11].

2.6.2. Accuracy and precision

Accuracy and precision tests shall be carried out at least three times. Each test was carried out using four QC concentrations, namely LLOQ, QCL, QCM, and QCH. Each concentration of QC samples was made as many as ≥5 replicas. Accuracy and precision testing can be done intraday (within-run accuracy) and between-day (between-run accuracy). The test shall meet the requirements of acceptance of the calibration curve, including LLOQ. This test does not have QC acceptance criteria. The acceptance requirement for accuracy testing is all concentrations ±15% of the minimum concentration, except LLOQ ±20%. The acceptance requirement for precision testing is %CV for all concentrations of ±15%, except LLOQ ±20%.

3. Results and discussion

3.1. Analysis method for determination of O^6-Methylguanine levels in DBS samples

This research requires a sensitive and selective method because the analyte compounds are at very few levels. The UPLC-MS/MS method can be used as a suitable analysis method because it can provide good results with repeatability that meets the criteria. We chose to use the analytical method developed by Vianney, Harahap, & Suryadi (2021) because the results of their research have been shown to provide fast, selective, and sensitive analysis results for O^6-Methylguanine. This method is also considered the most suitable compared to other O^6-Methylguanine analysis methods because the places and tools used are the same so that the validation does not have to be full validation, but only partial validation. Besides, the analysis method developed by Vianney, Harahap, & Suryadi (2021) has been optimized from various parameters, such as mass condition, mobile phase combination, mobile phase composition, flow rate, mobile phase gradient elution, and sample preparation and extraction process. The best results of that study were used as a method to perform the analysis in this study which can be seen in Chapter 2 of this study.

After the data of optimization has been collected, full validation was performed on the conditions above. Full validation was performed according to guidelines from the FDA. The LLOQ value obtained was 0.5 ng/mL with the %diff value ranged from -8.90% to 11.40% and the %CV value of 8.95%. The calibration curve made in 6 concentration levels, which was from a concentration of 0.5 ng/mL to 20 ng/mL, was linear and the correlation coefficient value was above 0.98. The %interference of analyte and Allopurinol obtained from the selectivity test consecutively was 8.64%–13.54% and 1.329–2.022%. The intraday accuracy value was ranged from 91.99 – 106.29% with %CV values of ≤4.61%, while the interday accuracy value was 96.23–109.45% with %CV values of ≤4.97%. The average recovery value of O^6-Methylguanine and Allopurinol was 82.62% and 81.10% respectively. The carryover value obtained from the test was 11.12%–12.65% for O^6-Methylguanine and 1.30%–1.68% for Allopurinol. The %diff obtained from the dilution integrity test was ranged from -3.70% to 14.62% with the %CV 2.82% for the 2QCH concentration, 6.39% for the QCH concentration, and 3.90% for the ½ QCH concentration. A little ion suppression from the matrix against the internal standard was found during the matrix effect test. The internal standard normalized matrix factors obtained were 1.07% for the QCL and 1.09% for the QCH concentration with the %CV value were 4.20% and 5.03% respectively. The O^6-Methylguanine and Allopurinol stock solutions were stable for 24 h at room temperature and 30 days in the refrigerator (-4 °C). All parameters tested in full validation have been approved and met the FDA guidelines acceptance criteria so that this method can be declared valid to be carried out on the same experiment at a later date. For these reasons, we chose the results of the method development from Vianney, Harahap, & Suryadi (2021) as a reference for conducting this research. The fragmentation spectrum of O^6-Methylguanine obtained from that study can be seen in Figure 2.

3.2. Partial validation

As explained in the previous section, all methods being used in this analysis, both sample preparation and analysis methods, followed the method developed by Vianney, Harahap, & Suryadi (2021), without any modification. Moreover, the laboratory, tools, and instruments being used in this research were also the same as Vianney, Harahap, & Suryadi (2021)'s. Therefore, only partial validation was carried out in this study with the purpose to ensure that the method used still met the requirements for accuracy and precision despite the changes in analyst. Based on FDA and EMA guidelines, partial validation can range from as little as the determination of within-run accuracy and precision, to almost full validation. The following is the partial validation that was carried out in this study before the analysis.

3.2.1. Calibration curve

The calibration curve was prepared using 6 concentrations in the range of 0.5–20 ng/mL. The linear regression obtained was $y = -0.005201842 + 0.261433828x$. The linearity value is indicated by the value of r, which was equal to 0.996293305. This shows that the linearity of the calibration curve is good enough because the r-value is above 0.99. The calibration curve graphic can be seen in Figure 3. The %diff value of LLOQ concentration was 19.85%, while the %diff of other concentrations was in the range of -2.76%–13.96%. This met the requirements set by the

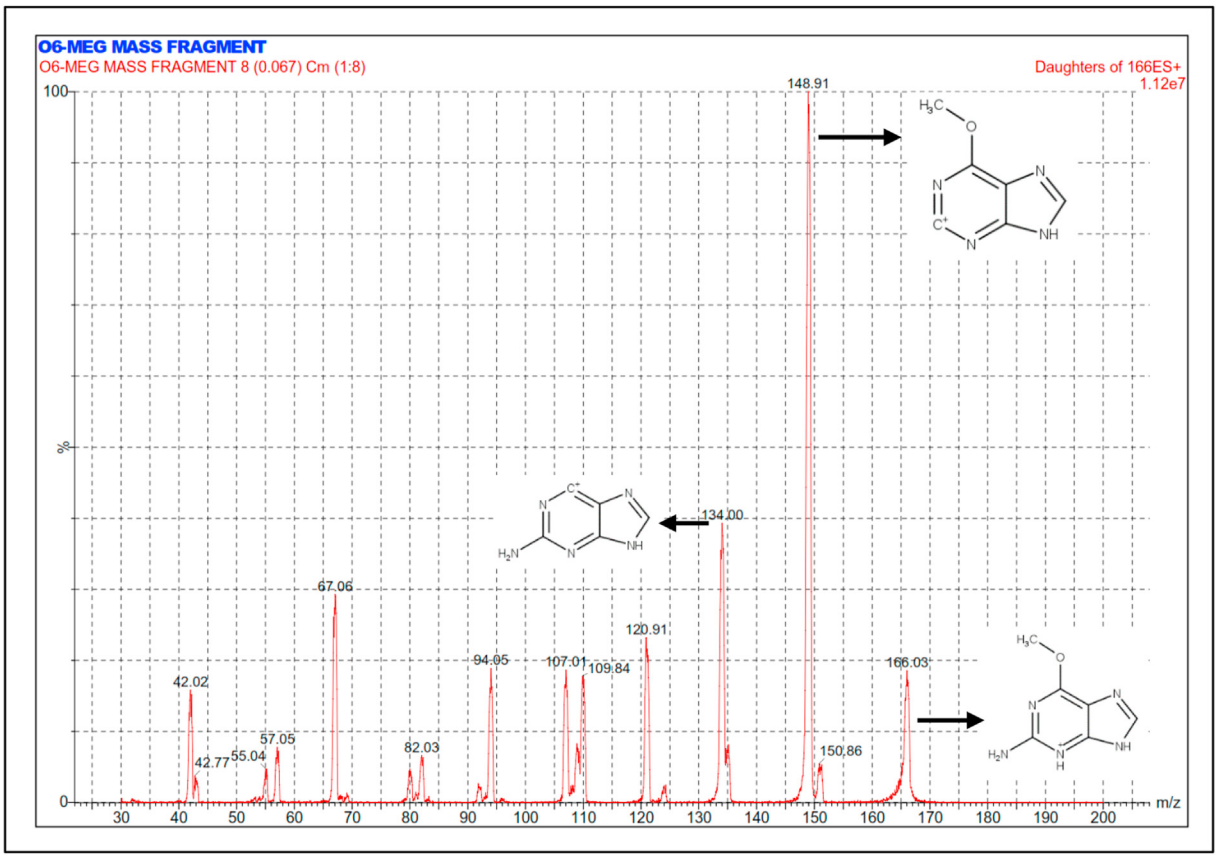

Figure 2. Fragmentation spectrum of O^6-Methylguanine (Vianney, Harahap, & Suryadi, 2021).

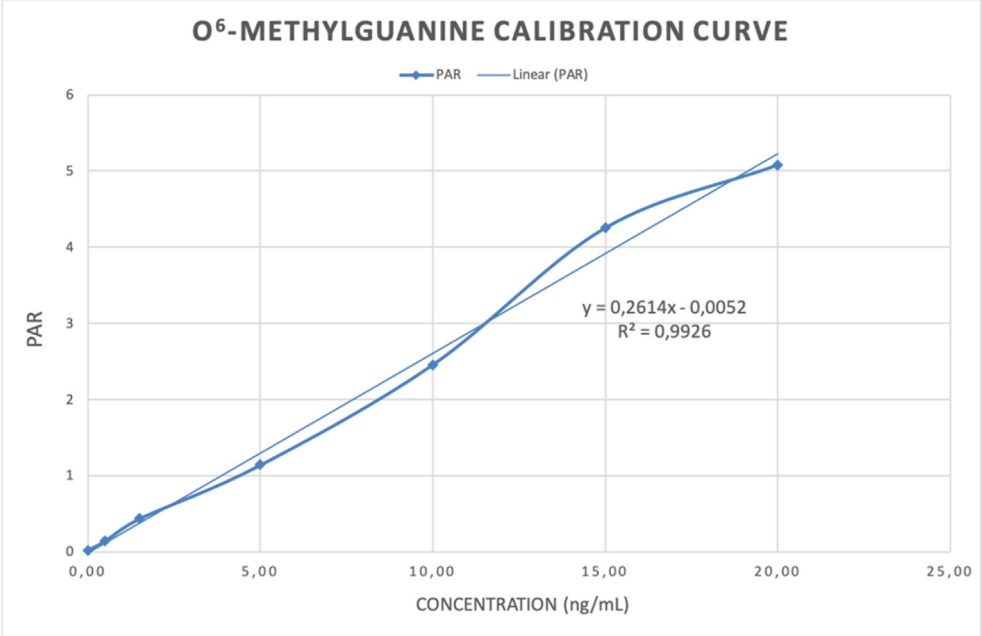

Figure 3. O^6-Methylguanine calibration curve.

FDA, which is the %diff for LLOQ should not be more than ±20%, while for other concentrations should not be more than ±15%.

3.3. Within-run accuracy and precision

Accuracy and precision were carried out using the concentrations of LLOQ, QCL, QCM, and QCH. Each concentration was made in 5 replicates. The test was carried out 3 times on the same day. The test results for accuracy and precision can be seen in Table 2. Based on the % diff value obtained, it can be concluded that the method met the requirements set by the FDA because there is no %diff value that exceeds ±20% in LLOQ, and the %diff value for the others also did not exceed ±15%. In addition, the proposed method is also precise because all of the % CV value obtained is no more than ±15%.

Table 2. Within run accuracy and precision of O^6-Methylguanine.

Concentration	1st run		2nd run		3rd run	
	Accuracy range (%diff)	Precision (%CV)	Accuracy range (%diff)	Precision (%CV)	Accuracy range (%diff)	Precision (%CV)
LLOQ	5.29–16.72%	4.63%	-5.49 to 6.63%	5.42%	-1.92 to 6.95%	3.26%
QCL	-0.06 to 6.49%	4.90%	-3.28 to 5.43%	4.17%	-5.99 to 5.26%	4.70%
QCM	-7.82 to 7.53%	5.91%	-5.91 to 8.17%	5.94%	-8.00 to 2.81%	4.71%
QCH	-4.31 to -0.01%	1.89%	-3.49 to 3.24%	4.13%	-4.73 to 3.38%	3.78%

3.4. Application of method in breast cancer patients

The method that has been developed by Vianney, Harahap, & Suryadi (2021) was applied to 16 breast cancer patients at the Dharmais Cancer Hospital. The inclusion criteria were patients receiving Cyclophosphamide as a chemotherapy regimen, either in single or in combination dose; each patient should not receive any other alkylating agents, the patient has gone through at least two chemotherapy cycles, and patients were willing to participate in the study and have signed informed consent. The chemotherapy cycles varied between 2 – 6. Samples were taken as the patient came to Dharmais Cancer Hospital to receive the therapy regimen. It was taken 30 min after the entire treatment regimen was fully administered.

The sampling time did not affect this study because this study aimed to analyze the levels of O^6-Methylguanine that had been formed and accumulated in the body due to the use of Cyclophosphamide in the previous cycle. Therefore, the amount of dose and number cycles that the patient has gone through were more important. The 30 min were chosen to keep the patients comfortable. If the blood sample was drawn while the patient was still receiving the therapy regimen, it would be more painful for them.

The 50 μL of blood samples were taken by finger-prick and spotted on DBS paper using a pipette to reduce the hematocrit effect. Then the sample was extracted using QIAamp DNA Mini Kit, isolated by dissolving the extract in a mixture of water and 90% formic acid and heated at 85 °C for 60 min.

The result showed that O^6-Methylguanine was detected and successfully quantified in all collected samples in the range of 0.55–6.66 ng/mL. The lowest concentration was found in a patient who had received FAC therapy for 3 cycles, while the highest one was found in a patient who had received FAC therapy for 5 cycles. All of the analysis results and O^6-Methylguanine concentration calculation from the samples are shown in Table 3 which indicates that all of the patients who received the Cyclophosphamide regimen, experienced accumulation of O^6-Methylguanine in their blood.

The result of this study was compared with the result obtained from the study carried out by Harahap, Andalusia, Crystalia, Nurfaradilla, & Harmita (2015). This study also analyzed the levels of O^6-Metilguanin in the blood of cancer patients who received cyclophosphamide in their treatment regimen. We used this research as a comparison to our research because it took place in the same hospital, Dharmais Cancer Hospital, so it uses the same therapeutic regimen system. Besides, research on O^6-Methylguanine in the blood of cancer patients has never been done in other hospitals before. The sample used in their study was blood samples from veins (venipuncture method). Analysis was performed using UPLC-MS/MS, column C18 Acquity® UPLC BEH (1.7 μm, 2.1 × 100 mm), mobile phase consisted of acetic acid 0.05% in aquabidest - acetonitrile (95:5), isocratic elution in 3 min, flow rate of 0.3 mL/min, ionization method was ESI +, quantification traces 166.1 > 149.1 and 166.1 > 134.1, injection volume was 10.0 μL. O^6-Methylguanine's peak showed in 1.46 min. Extrapolation from calibration curve data gave LOD 1.05 ng/mL and LOQ 3.50 ng/mL. Among 72 samples analyzed, O6-Methylguanine was detected in 17 samples and could be quantified in 1 sample in a concentration of 5.8680 ng/mL [5].

Based on the results of both studies, both of the method have the same LLOQ value, which is 0.5 ng/mL, but the amount of sample needed in the method used in this study was less than the method used by Harahap, Andalusia, Crystalia, Nurfaradilla, & Harmita (2015). Moreover, our study has successfully detect and quantify all of the samples, thus the method used can be considered more selective, accurate, and precise. Also, the biosampling method with DBS is less invasive and more comfortable for the patients, so it is more applicable than the other analysis method of O^6-Methylguanine in blood samples.

The formation of O^6-Methylguanine is preceded by the oxidation of Cyclophosphamide by the P450 enzyme to form 4-hydroxycyclophosphamide (4-OHCP) metabolites. The presence of 4-OHCP is also offset by the presence of Aldophosphamide which can open the tautomeric bonds. Then, 4-OHCP and Aldophosphamide enter the cells. Aldophosphamide is decomposed into active alkylating metabolites, namely Phosphoramide mustard and acrolein (a by-product with less activity) [12]. This

Table 3. Data and analysis result of breast cancer patients.

Patients	Age (years)	Chemotherapy	Doses	Cycle	Measured value (ng/mL)
SN 1	54	FAC	875/85/875	5	4.33
SN 2	55	FAC	800/80/800	6	4.47
SN 3	31	FEC	900/90/900	3	5.29
SN 4	39	TAC	114/90/900	2	5.18
SN 5	46	FEC	900/144/900	5	3.71
SN 6	37	FAC	740/74/740	5	2.98
SN 7	28	AC	100/1000	6	6.61
SN 8	63	TC	130/803	2	1.07
SN 9	33	FAC	750/75/750	3	0.55
SN 10	52	AC	94/940	4	3.05
SN 11	51	FAC	775/77/775	5	0.97
SN 12	43	FAC	750/73/750	5	6.66
SN 13	46	FAC	700/70/700	3	0.76
SN 14	52	FEC	900/90/900	4	0.97
SN 15	33	FAC	920/92/920	5	0.70
SN 16	44	AC	60/600	6	0.67

active Phosphoramide mustard then alkylates the O and N positions of the nitrogenous base. Alkylation at the O^6 position on the guanine base produces O^6-Methylguanine which is mutagenic. The formation of this DNA adduct will inhibit the separation of DNA strands during the replication process and cause damage to DNA [13]. If the DNA damage is not repaired, it can cause mutations and cancer over time [14].

However, by seeing this analysis result, it cannot be concluded that the higher the dose and the longer the duration of therapy, the greater the concentration of O^6-Methylguanine produce. The O^6-Methylguanine concentration found in patients who received FEC therapy for 3 cycles was greater than in those who received FAC for 6 cycles. This might occur due to the differences in O^6-Methylguanine-DNA-methyltransferase (MGMT) enzyme ability to repair DNA damaged [15].

The DNA repair was done by transferring the methyl group in the O^6-guanine position to cysteine residues, thus prevent gene mutations, cell death, and tumorigenesis due to alkylating agents. Because of this mechanism, the concentration of O^6-Methylguanine formed by alkylating agents, such as Cyclophosphamide, can be reduced. Consequently, the O^6-Methylguanine concentration is greatly influenced by the ability of MGMT to repair DNA damaged [15].

Hence, further researches were needed to determine the minimum concentration of O^6-Methylguanine which can lead to secondary cancer. By these follow-up studies, the data obtained in this study can be better interpreted and correlated with the patients receiving Cyclophosphamide's possibilities in developing secondary cancers.

4. Conclusion

This study was carried out by using the method developed by Vianney, Harahap, & Suryadi (2021) to analyze O^6-Methylguanine in breast cancer patients who received Cyclophosphamide regimen therapy. This method has been partially validated and met the criteria set by the FDA. O^6-Methylguanine was detected and quantified in all of the patients who participated in this study (16 patients), in the range of 0.55–6.66 ng/mL. This indicates that O^6-Methylguanine accumulation in cancer patients receiving the Cyclophosphamide regimen is very likely to occur. The larger the accumulation of O^6-Methylguanine, the greater the chance of patients developing secondary cancer. Therefore, it is necessary to do therapeutic drug monitoring (TDM) in this group of patients to reduce the possibility. The method used in this study has the potential to be applied on a larger scale for the implementation of TDM in cancer patients.

Declarations

Author contribution statement

Yahdiana Harahap: Conceived and designed the experiments.

Athalia Theda Tanujaya; Farhan Nurahman; Aurelia Maria Vianney: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Denni Joko Purwanto: Contributed reagents, materials, analysis tools or data.

Funding statement

This work was supported by the Directorate of Research and Community Services Universitas Indonesia, Depok, Indonesia.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

This study was reviewed and approved by the Research Ethics Committees of "Dharmais" Cancer Hospital, Jakarta 11420, Indonesia (No.023/KEPK/II/2020).

References

[1] Prevention, C. for D. C, What Is Breast Cancer?, 2018. Retrieved from, https://www.cdc.gov/cancer/breast/basic_info/what-is-breast-cancer.htm.

[2] E.S. El. Desoky, Pharmacogenomics And/or Therapeutic Drug Monitoring in Cancer Chemotherapy Are Used Independently or Together for Dose Optimization. *Pulsus*, 2018. Retrieved from https://www.pulsus.com/scholarly-articles/pharmacogenomics-andor-therapeutic-drug-monitoring-in-cancer-chemotherapy-are-used-independently-or-together-for-dose-optimization-4763.html.

[3] L.H. Swift, R.M. Golsteyn, Genotoxic anti-cancer agents and their relationship to DNA damage, mitosis, and checkpoint adaptation in proliferating cancer cells, Int. J. Mol. Sci. 15 (3) (2014) 3403–3431.

[4] B. Ma, I. Stepanov, S.S. Hecht, Recent studies on DNA adducts resulting from human exposure to tobacco smoke, Toxics 7 (1) (2019).

[5] Y. Harahap, R. Andalusia, Y. Crystalia, S. Nurfaradilla, Harmita, Analysis of O^6-Methylguanine in cancer patient blood during administration of cyclophosphamide using ultra-high-performance liquid chromatography-tandem mass spectrometry, J. Adv. Med. Pharmaceut. Sci. 2 (1) (2015) 20–28.

[6] A. Paci, G. Veal, C. Bardin, D. Levêque, N. Widmer, J. Beijnen, E. Chatelut, Review of therapeutic drug monitoring of anticancer drugs part 1 - Cytotoxics, Eur. J. Cancer 50 (12) (2014) 2010–2019.

[7] M. Broto, R. McCabe, R. Galve, M.-P. Marco, A high-specificity immunoassay for the therapeutic drug monitoring of Cyclophosphamide, Analyst 144 (17) (2019) 5172–5178.

[8] Y. Harahap, A.M. Vianney, H. Suryadi, Method development and validation for measuring O^6-Methylguanine in dried blood spot using ultra high-performance liquid chromatography tandem mass spectrometry, Drug Des. Dev. Ther. 15 (2021) 963–971.

[9] L. Lennard, Therapeutic drug monitoring of antimetabolic cytotoxic drugs, Br. J. Clin. Pharmacol. 47 (2) (1999) 131–143.

[10] QIAGEN, QIAamp DNA Mini and Blood Mini Handbook, Forest Stewardship Council, Hilden, 2016.

[11] Food and Drug Administration, Bioanalytical Method Validation Guidance for Industry Bioanalytical Method Validation, FDA Guidance for Industry, 2018 (May), 1–22. Retrieved from http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm and/or http://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/default.htm.

[12] K.A. Stroda, J.D. Murphy, R.J. Hansen, L. Brownlee, E.A. Atencio, D.L. Gustafson, S.E. Lana, Pharmacokinetics of cyclophosphamide and 4-hydroxycyclophosphamide in cats after oral, intravenous, and intraperitoneal administration of cyclophosphamide, Am. J. Vet. Res. 78 (7) (2017) 862–866.

[13] S. Ganesan, A.F. Keating, Phosphoramide mustard exposure induces DNA adduct formation and the DNA damage repair response in rat ovarian granulosa cells, Toxicol. Appl. Pharmacol. 282 (3) (2015) 252–258.

[14] J.E. Klaunig, L.M. Kamendulis, B.A. Hocevar, Oxidative stress and oxidative damage in carcinogenesis, Toxicol. Pathol. 38 (1) (2010) 96–109.

[15] W. Yu, L. Zhang, Q. Wei, A. Shao, O^6-Methylguanine-DNA methyltransferase (MGMT): challenges and new opportunities in glioma chemotherapy, Front. Oncol. 9 (January) (2020) 1–11.