Use of Surrogate Samples to Monitor pH During High dose Methotrexate Therapy

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Abstract High dose methotrexate (Mtx) therapy is commonly used in hemato-oncological practice. Alkalization of urine is a part of high dose methotrexate therapy for preventing crystallization in urine to avert renal insufficiency. Alkalization is monitored by urine pH at regular intervals. Oral pH has occasionally been used as a surrogate for oral mucositis. To compare and correlate pH of various body secretions (venous blood, oral salivary, lacrimal and urine) among patients undergoing alkalization with intravenous sodium bicarbonate during high dose methotrexate therapy. A prospective single center study in patients with hematological malignancies receiving Mtx > 1.5 g/m² over 4–24 h. Patients were assessed for pH (from all 4 body fluids) at regular time intervals (q8 h) starting 6 h-prior and 48 h-

post initiation of Mtx therapy. Mean pH of urine/oral was compared to surrogate samples. The mean oral pH was 6.9 (SD 0.65), the mean urinary pH was 7.59 (SD 0.773) the mean pH by venous blood gas analysis (venous pH) was 7.388 (SD 0.059), the mean lacrimal pH was 7.4536 (SD 0.527). Repeated measures ANOVA suggests that pH of different body fluids differ and cannot be used interchangeably [$F(2.417, 309.361) = 54.89, p < 0.0005$]. There was no statistically significant correlation between any other pair of assessed body fluids. On paired t test only the means of venous pH and urinary pH did not differ statistically ($p 0.056$). Venous pH significantly correlated with urinary pH but the strength of correlation was weak ($r 0.184; p 0.037$). pH of different body fluids is statistically different even when sampled simultaneously thus the pH of one fluid cannot be substituted for other. Based on this study we cannot substitute urinary pH with any other body fluids presently in patients undergoing high dose methotrexate and alkalization except in rare circumstances when venous pH can be used as a poor surrogate for urinary pH in situations where urinary pH cannot be monitored due to any reason. There was no surrogate for oral pH among the studied body fluids.

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Introduction

Methotrexate is extensively used in the management of multiple hemato-oncological malignancies [1]. The side effect profile of Mtx is related to the dose administered. Clinically dosing is thus classified into high (>500 mg/m²), intermediate (50–500 mg/m²) and low dose (<50 mg/m²). High dose methotrexate (HD-Mtx) has a wide spread role in oncological practice starting from

solid tumors (e.g., osteosarcoma, leptomeningeal metastases) to hematological malignancies (central nervous system (CNS) prophylaxis in patients with leukemia, high-risk lymphoma, and primary CNS lymphoma). The major adverse effects secondary to HD-Mtx therapy include raised transaminases, oral mucositis, and renal insufficiency. Urinary and oral pHs are frequently monitored during HD-Mtx therapy for monitoring urinary alkalization and for an experimental role in predicting/evading oral mucositis respectively. We aimed at the use of surrogate samples to monitor pH during high dose methotrexate therapy.

It is mandatory to check urinary pH at periodic intervals during HD-Mtx therapy and urinary pH reflects the adequacy of alkalization of urine during HD-Mtx therapy. Alkalization of urine helps to dissolve methotrexate and prevents crystallization in the urinary tract, thus aid to avert renal insufficiency. There are some inherent bed-side problems with urinary pH monitoring. As patients are generally not catheterized the frequency of urinary pH evaluation shall, in turn, depend on the frequency of micturition. Inability to monitor pH at definite intervals frequently hinders the initiation or continuation of therapy awaiting therapeutic urinary pH ($>7-7.5$). These delays/interruptions in HD-Mtx therapy have financial implications by increasing hospital stay and can have a significant bearing on the efficacy of the therapy. Also, patients with renal tubular disorders can have concentration defects and alterations in urinary pH, situations wherein it is difficult to monitor urinary pH as a marker for alkalization. There are no studies to evaluate the efficacy of other body fluids pH as a substitute for urinary pH.

Oral pH has been used as a marker for predicting oral mucositis in various head and neck malignancies. Also, there are studies on the prevalence of oral mucositis in patients with low oral pH receiving radiation/high dose chemotherapy [2–6]. Also oral mucositis is a common complication in patients on HD-Mtx therapy related mainly to the duration of exposure vis-à-vis Mtx concentration [7–9]. There is a lack of standardization in checking oral pH, thus, it is important to search for alternative biological methods which can act as a surrogate for oral pH. So, it was hypothesized if urinary pH monitored routinely in HD-Mtx therapy can be substituted for oral pH or if any other body fluid can be used as a common surrogate for both urinary and oral pH. We selected venous blood pH measurement as one of the comparison parameters because blood gas analysis currently is the most standardized method of pH monitoring. Lastly, the reason for including lacrimal fluid is the perennial/easy availability of lacrimal fluid for analysis and previously studied role of lacrimal pH in multiple local and general settings [10].

With the given background, we conducted this prospective study to evaluate if other body fluids can be used as a surrogate for urinary/oral pH.

Aim and Objectives

The aim of the study was to assess the utility of surrogate samples to monitor pH among patients with hematological malignancies undergoing alkalization with intravenous sodium bicarbonate during HD-Mtx therapy. The objectives were to compare the pH of four different body fluids (i.e., urine, oral, lacrimal and venous blood) both in multivariate analysis/paired parameters and to assess their inter-correlation.

Methodology

This is a prospective systematic single center non-interventional study. It was carried out in hematology high dependence unit at a tertiary care center from North India. Informed consent was taken from all the patients prior to inclusion in the study based on Helsinki declaration.

Inclusion Criteria

(a) Patients receiving high dose methotrexate therapy (HD-Mtx in this study defined as any dose of methotrexate $>1.5 \text{ g/m}^2$ given over 4–24 h) (b) Age ≥ 12 years and ≤ 60 years. (c) Creatinine clearance (CrCl) $>60 \text{ ml/min}$ calculated by Cockcroft-gault equation (d) Absolute neutrophil count more than $1500/\mu\text{l}$ at the start of therapy.

Exclusion Criteria

(a) Having oral infections/dental caries and poor oral hygiene as assessed by the treating physician (b) Any clinical or microbiological evidence of UTI (c) Conjunctivitis or eye infections as assessed by the treating physician (d) Any clinical, radiological or microbiological evidence of systemic infections other than UTI/eye infections (e) Renal dysfunction (defined as CrCl $<60 \text{ ml/min}$ calculated by Cockcroft-gault equation) (f) Systemic disorders (e.g., diabetes, hypertension, hyperthyroidism).

High dose Methotrexate Therapy and Alkalization

HD-Mtx in this study has been defined as the therapeutic dosage of more than 1.5 g/m^2 of methotrexate in any hemato-oncological condition (leukemia and lymphoma). Urinary alkalization was achieved using intravenous sodium bicarbonate (*supplement 1—proforma for HD-Mtx*

in lymphoblastic leukemia, supplement2—proforma for HD-Mtx in primary CNS lymphoma).

pH Evaluation

We used semi-quantitative measurement for checking oral, urine and lacrimal pH using pH strips/litmus paper using colorimetric assay, whereas venous pH was based on venous blood gas (VBG) analysis. The primary reason for using pH color strips vis-à-vis pH meter was to mimic bedside practices in research scenario and make the study findings more relevant in day-to-day clinical settings. To ensure standardization and accuracy, the pH strips were randomly counterchecked with standard commercially available pH solutions.

pH evaluations of all four body fluids were done simultaneously within a time interval of 10 min to avoid temporal changes in acid base status. The pH evaluation was started 6 h before high dose methotrexate therapy and continued during alkalization at regular intervals (q8 h) for 48 h post initiation of HD-Mtx therapy as part of this investigational protocol. Samples of 34 patients at these regular time intervals were sampled for these four parameters simultaneously.

pH Strips and its Readings

pH strips and color coding for different values is depicted in Fig. 1a. The colorimetric card has read from 6 to 14 (in

whole numbers). Using visual analysis, the pH value is decided on a comparison of the color of the strip with the colorimetric card. If the color of the strip was not appropriating to any specified whole number value, an intermediate value was given. All pH evaluations were done by the first author and reconfirmed by the second author simultaneously, in the case of discrepancy in values a consensus decision was taken.

Urinary pH Measurement

To check urine pH a single strip was dipped in freshly passed midstream urine collected in a sterile container. It was allowed to dry for at least 20 s and then the color of the strip was compared with the colorimetric card.

Oral pH Measurement

Oral pH was checked by keeping the strip in the sublingual area for few seconds. The pH readings were obtained in the same manner as described above (Fig. 1b—technique for seeing oral pH). Patients abstained from oral intake of solids or liquids for 30 min before the pH monitoring.

Lacrimal pH Measurement

For lacrimal pH measurement, the tip of the pH strip was folded and was held close to the eye for some time for initiating tear production. The tip was then dipped in the

Fig. 1 **a** pH strips and the colorimetric comparison card. **b** Procedure for checking oral pH. **c** Procedure for checking lacrimal pH

pocket of the eye to allow the corner of the strip to get wet with that fluid and then a change of color was compared with the colorimetric card as described above (Fig. 1c—technique for seeing lacrimal pH). The method used was akin to the validated Schirmer's test used for evaluating dry eyes.

Venous pH Measurement

Venous pH was assessed using venous blood samples either from radial or CVC sample. In the case of CVC sample, a thorough flushing of the catheter was carried out prior to sampling. Also, the first 5 ml was discarded to avoid pre-analytic variability in the analysis. The samples were conducted on dry ice to the blood gas analyzer and were analyzed immediately to avoid time dependent change in pH.

Statistical Analysis

We used SPSS Ver.16.0 for statistical analysis. Comparison of means was done using repeated measures ANOVA, whereas paired-t test was used to compare the means of paired samples. Correlations were assessed using Pearson's correlation coefficient ($p < 0.05$ was considered significant).

Results

A total of 180 simultaneous samples for all four parameters were collected from 34 patients. (Flow diagram—Fig. 2). 151 samples were finally analyzed (of which 21 venous blood gas analyses samples with time to analysis >10 min were excluded during relevant statistical exercises).

Fig. 2 Flow diagram demonstrating sample selection

The median age of patients was 21 years (mean 23.26 years; range 13–65 years, SD 11.67 years). The study cohort included 11 females and 23 males. Median dosage of HD-Mtx given in our included patients 3.0 g/m² (mean 2.8 g/m², range 1.5–5 g/m², not <1.5 g/m² in any patient). It included 27 patients of ALL (26 modified BFM protocol CNS prophylaxis, 1 CALGB protocol), three patients of NK-T Cell (SMILE protocol) and four patients with primary CNS lymphoma (De-Angelis protocol).

The mean oral pH was 6.9 (SD 0.65) (n 151), the mean urinary pH was 7.59 (SD 0.773) (n 151) the mean pH by venous blood gas analysis (venous pH) was 7.388 (SD 0.059) (n 130), the mean lacrimal pH was 7.4536 (SD 0.527) (n 130) (Table 1). A repeated measures ANOVA (N 34 patients, n 129 simultaneous samples) with a Greenhouse–Geisser correction determined that mean pH concentration differed statistically significantly between the four modalities [F (2.417, 309.361) = 54.89, $p < 0.0005$]. Post hoc tests using the Bonferroni correction used revealed the following: (a) Mean oral pH (6.83 ± 0.6) was higher than pH from all other measured body fluids ($p < 0.0005$). (b) Mean Urine pH (7.65 ± 0.79) was higher than mean venous pH assessed by VBG (7.39 ± 0.06) ($p < 0.001$) and mean lacrimal pH (7.49 ± 0.52) ($p = 0.257$). (c) Mean venous pH was lower than mean lacrimal pH which was not statistically significant ($p = 0.149$).

Analysis of the repeated measures ANOVA suggests that pH of different body fluids differ and cannot be used interchangeably which could be explained owing to the different handling of the acid base excess by different tissues.

Paired t test to compare means of different paired samples is depicted in Table 1. Other than venous blood and urinary pH, there were significant differences in means among all other pairs of assessed body fluids when compared by the paired-t test.

Table 1 Paired t test to compare means of different paired samples

Pair 1	Urinary pH	Oral pH	$p < 0.0001$
	Mean 7.59 (n 151)	Mean 6.9 (n 151)	
Pair 2	Urinary pH	Venous pH–VBG	$p \ 0.056$
	Mean 7.6512 (n 130)	Mean 7.38857 (n 130)	
Pair 3	Urinary pH	Lacrimal pH	$p < 0.0001$
	Mean 7.59 (n 151)	Mean 7.45 (n 151)	
Pair 4	Oral pH	Lacrimal pH	$p < 0.0001$
	Mean 6.9007 (n 151)	Mean 7.4536 (n 151)	
Pair 5	Venous pH–VBG	Lacrimal pH	$p \ 0.027$
	Mean 7.38867 (n 130)	Mean 7.4923 (n 130)	
Pair 6	Oral pH	Venous pH–VBG	$p < 0.0001$
	Mean 6.8385 (n 130)	Mean 7.38867 (n 130)	

Statistically not significant value is in bold

VBG venous blood gas analysis

Table 2 Pearson's correlation of pH from different body fluids with *p* values

Pairs			Pearson's correlation coefficient	<i>p</i> value
Pair 1	Urinary pH	Oral pH	0.158	0.054
Pair 2	Urinary pH	Venous pH-VBG	0.184	0.037
Pair 3	Urinary pH	Lacrimal pH	0.098	0.235
Pair 4	Oral pH	Lacrimal pH	−0.028	0.732
Pair 5	Venous pH-VBG	Lacrimal pH	−0.024	0.784
Pair 6	Oral pH	Venous pH-VBG	−0.666	0.453

Statistically significant values are in bold

VBG venous blood gas analysis

On Pearson's correlation only urinary and venous blood pH had a statistically significant correlation (*p* 0.037), though the strength of correlation as measured by Pearson's correlation coefficient was very weak (*r* = 0.184). There was no statistically significant correlation between any other pair of samples (Table 2; Fig. 3a–f).

Discussion

pH in various body fluids: An individual at a given moment can have different pH in varying body fluids owing to the differential distribution of H⁺ ionic pumps and HCO^{3−} diffusion in cells. This also explains why the same individual develops differential organ involvement and variable complications despite same therapy. Studies have proven relation of organ specific pH and its pathological prevalence [10, 11]. Further studies for therapeutic interventions to increase pH of specific body fluids can help to ameliorate the side effects of drugs [4, 12, 13]. We during the study tried to compare and correlate urinary pH with lacrimal, oral pH by semi-quantitative methods and venous blood pH assessed by blood gas analysis. The reason for selecting the above surrogates is an easy availability of these samples. Oral, urine, and lacrimal pH were based on semi-quantitative technology using pH strips/litmus paper using colorimetric assay. Despite the availability of the pH meter to check urine pH we choose to use color pH strips for making the study more practical and clinically relevant (to mimic bed side practices). Also, salivary/oral and lacrimal pH is very difficult to be evaluated by the pH meter. We selected venous blood over arterial blood for blood-gas analysis to avoid more traumatic and complication prone procedure repeatedly. During evaluation for lacrimal pH, no patient developed conjunctivitis or ocular complications secondary to the strips.

The Relevance of Urinary pH and Correlation with Blood pH

The potential for methotrexate to precipitate in renal tubules particularly in HD-Mtx during treatment of

hemato-oncological conditions led to several studies on urine alkalization or clearance of methotrexate. Urinary alkalization (previously termed as forced alkaline diuresis or alkaline diuresis) is defined as administration of intravenous sodium bicarbonate to produce urine with pH ≥ 7.5. Position paper on urine alkalization suggested an inevitable increase in blood pH (alkalemia) approaching 7.7 on the administration of bicarbonate [11]. In our study the mean blood pH was lower being 7.38 (SD 0.06), this could be attributed to few samples taken before achieving therapeutic urinary alkalization. On the exclusion of such recordings when urinary pH < 7.5, the mean blood pH was 7.67 (SD 0.12). Raymond et al. studied the effect of urinary alkalization on salicylate elimination in dogs, they did not find any correlation between blood and urinary pH [12]. There have been no studies to show the exact linear correlation between urinary pH and blood pH. In our study, the mean pH of blood and urine was significantly different and we also could not find any significant linear correlation between blood and urinary pH.

Salivary pH and its Correlation

Sepet et al. [13] studied the relevance of salivary pH in relation to dental health in patients with acute lymphoblastic leukemia on maintenance methotrexate therapy. Rojas de Morales et al. [14] studied pH and salivary sodium bicarbonate during administration protocol methotrexate in children with leukemia. They systematically studied pH at baseline, 12 h after intravenous administration of sodium bicarbonate (before administering methotrexate) and 3 h after administering methotrexate (time of maximum concentration). There was no variation in salivary pH during alkalization (neutral pH throughout the protocol) contrary to Ertugrul et al. [15] and Al-Nowaiser et al. [16]. In our study we assessed salivary pH at random time points during a similar HD-Mtx protocol with the mean pH being 6.71 (SD 0.42) similar to the above study. They concluded by suggesting studies to evaluate the relationship between blood and salivary pH in patients undergoing alkalization during high dose methotrexate therapy which formed the basis of our study. In our study,

Fig. 3 Correlation between pH of various body fluids (a–f)

the mean pH of blood and oral cavity was significantly different and we could not find any correlation between blood and oral pH. Zambrano et al. [17] studied various

local factors such as dental caries, plaque level, and gingivitis which increased with age and produces a qualitative change in the microbial flora of saliva and

induces a decrease in salivary pH. The study by Rojas de Morales et al. [14] was confounded by the lack of evaluation of local factors which could have confounded their findings contrary to our study wherein we excluded all patients with presence of local (oral) confounding factors.

Strengths and Limitations of the Study

This is the first of its kind wherein the pH of various body fluids was assessed in a hemato-oncological case scenario, with no similar study to date. The major limitation of the study was a lack of arterial blood gas analysis to understand the actual acid base status of the body. Use of semi-quantitative pH analysis is strength in a way (that it mimics daily clinical practice) and limitation in another way (that it precludes the actual measurement of the pH by using more sensitive/standardized methods). Also, the ideal way of monitoring the HD-Mtx therapy is by Mtx serum levels. Due to the lack of local facility in monitoring Mtx levels, lack of standardization and the cost involved, we haven't monitored the Mtx levels as part of the study. Monitoring Mtx levels would have added strength to the study particularly the correlation of various fluid pH and methotrexate levels.

Conclusion

pH of different body fluids is statistically different even when sampled simultaneously thus the pH of one fluid cannot be substituted for other. Also, there is no statistically significant correlation of commonly measured urinary pH with any other body fluids (except venous blood pH, that too with very weak correlation) thus undermining the importance of local factors and kidney in handling the acid base load of the body. Based on this study we cannot substitute urinary pH with any other body fluids presently in patients undergoing high dose methotrexate and alkalization except in rare circumstances when venous pH can be used as a poor surrogate for urinary pH in situations where urinary pH cannot be monitored due to any reason. There was no surrogate for oral pH among the studied body fluids.

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Compliance with Ethical Standards

Conflict of interest All authors declare that there is no conflict of interest.

Human and Animal Rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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