


Salivary immunoglobulin A level during steroids and chemotherapy treatment administered in remission induction phase among pediatric patients with acute lymphoblastic leukemia

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Abstract

The agents used in the treatment of acute lymphoblastic leukaemia (ALL) might affect the oral health of cancer patients.

The study aims to assess the changes in the levels of immunoglobulin A (IgA) in saliva and blood, during first 22 days of intensive chemotherapy of ALL in children.

Saliva and blood samples were taken from 24 patients, including 13 boys and 11 girls (age range: 4–17 years) on days 1, 8 and 22 of treatment. The levels of immunoglobulin A and total protein were estimated in samples at each time-point. The distribution of the quantitative variables was assessed using the Shapiro-Wilk test. Non-parametric statistics were used to compare the levels of repeated measurements and post hoc non-parametric analysis was applied for between time-point comparisons.

A constant relationship was found between the levels of Ig A in blood and saliva ($r=0.28$; $P=.031$). No change in salivary IgA level was observed in the prednisone-only prephase, but it dropped significantly on day 22 (10.7 ± 4.8 vs 9.6 ± 6.4 vs 5.7 ± 3.9 ng/mL; $P=.04$), when chemotherapy was given (anthracycline, vincristine, L-asparaginase).

In blood, the total protein level decreased significantly between day 1 and 22 (6.2 ± 0.4 vs 5.1 ± 0.3 g/dL; $P=.001$). Lymphocyte count (per microliter) also decreased (2.12 ± 0.8 vs 0.41 ± 0.1 vs 1.08 ± 0.5 ; $P=.002$). Four children suffered from oral mucositis graded 1 or higher between days 8 and 22.

Chemotherapy given during the treatment of childhood ALL is associated with a reduction in the level of salivary immunoglobulin A. Prevention of the drop of salivary IgA may diminish the risk of occurrence of acute mucosal complications.

Abbreviations: ALL = acute lymphoblastic leukaemia, IgA = immunoglobulin A.

Keywords: acute lymphoblastic leukaemia, children, immunoglobulin A, mucositis, saliva

1. Introduction

Saliva is mostly composed of water (99.5%) with electrolytes, mucus, white blood cells, epithelial cells, glycoproteins, enzymes such as amylase and lipase, and antimicrobial agents including lysozyme or lactoferrin.^[1] It has been demonstrated that the

concentrations of a number of salivary constituents can vary to a great degree, and the overall composition of saliva is influenced by the general health and the medications taken by a patient.^[2–4] These components are responsible for the so-called nonspecific mechanism, which are known to neutralize the pathogens. The

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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. The study was approved by the Ethical Committee of the Medical University of Lodz (IRB number: RNN/35/13/KE).

Informed consent was obtained from all individual participants and their care givers included in the study.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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specific defence reaction is based on cellular (T-lymphocytes) and immunoglobulin-mediated responses, among which salivary Immunoglobulin A (IgA) seems to be most important.^[5] The levels of IgA were found to be related to the oral states during e.g. recurrent aphthous stomatitis or primary Sjogren's syndrome.^[6,7]

Anti-cancer therapy, especially in paediatric population, affects the dental health.^[8,9] The most common cancer in childhood is acute lymphoblastic leukaemia (ALL). Intensive treatment of ALL using chemotherapy may generate severe immunosuppression, which may lead to many complications including mucositis, a painful inflammation and ulceration of mucosa lining the gastrointestinal tract. The oral cavity is a common site of mucositis, which temporarily prevents patients from chewing, swallowing or even speaking normally.^[10,11] In many current ALL therapeutic regimens, an aggressive anti-cancer therapy during the first weeks after diagnosis is crucial for the clinical outcome of the patients, but is also associated with many complications including mucosa lesions.

Since a link between mucosal immunity and anti-cancer treatment is still not fully understood, the aim of our study was to determine the changes in the levels of immunoglobulin in saliva and serum in paediatric patients suffering from ALL, during the first twenty-two days of anticancer therapy.

2. Materials and methods

2.1. Study group

The inclusion criteria comprised an age below eighteen, newly diagnosed ALL and a written consent for examination from parents or care givers. In addition, it was important that the child was willing to take part in the investigation. Over the course of the 18-month study, thirty-seven children diagnosed with ALL were included in the investigation. Of these, 2 refused to participate, 3 were unable to spit in the tube, 1 was excluded because of Down syndrome, 2 were referred to other hospitals and 1 was lost. In addition, a further 4 were excluded due to samples of saliva being missed or omitted. A final study group consisted of 24 children (13 boys and 11 girls) diagnosed "de novo" with ALL based on cytological, flow cytometric and cytogenetic evaluation of the bone marrow according to the current treatment protocol (ALL-IC2009) approved by the Polish Paediatric Leukaemia Lymphoma Study Group.^[12] All children were diagnosed and treated in 1 Paediatric Oncology Unit in

2018 or 2019. The patients were aged between 4 and 17 years (median 10.1 years). Their details are presented in Table 1. The study was approved by the Ethical Committee of the Medical University of Lodz (IRB number: RNN/35/13/KE).

2.2. Treatment protocol

All patients were treated according to the ALL-IC2009 treatment protocol for lymphoblastic leukaemia in children. During the first 7 days, only systemic prednisone therapy was given (60 mg per m² body surface area); this was followed by daunorubicin (30 mg/m²/d), vincristine (1.5 mg/m²/d) and *E coli* L-Asparaginase (5000 U/m²/d) (Medac/KYOWA, Germany) during the next twenty-six days, regardless of the stratification to risk group. The daunorubicin and vincristine were delivered intravenously on days 8 and 15. The vincristine dose was repeated again on days 22 and 29. On day 11, L-asparaginase was introduced intravenously; the doses were repeated 7 times, every 3 days (Fig. 1).

2.3. Collecting samples and laboratory testing

Saliva and blood samples were taken at 3 time points: on the first, eighth and 22nd day of treatment (Fig. 1). The saliva was collected in the morning, before breakfast and before chemotherapy was given. Each time, about 5 ml of saliva was collected with sterile tubes, then preserved with aprotinin, centrifuged (2000 rpm for 10 minutes) and then stored at -80 degrees Celsius.

In the salivary samples, the levels of immunoglobulin A (with use of Human IgA Platinum ELISA BMS by eBioscience) and total protein (with use of TP0300-1KT-total protein kit by SIGMA, Germany) were tested. The levels of immunoglobulin A and total protein in blood were also checked in a clinically-certified diagnostic laboratory using a Cobas INTEGRA device (Roche).

All symptoms that occurred during therapy were recorded. Adverse events (AEs) were assessed and graded with the use of the Common Terminology Criteria for Adverse Events (version 4.0).^[13]

2.4. Statistical evaluation

The distribution of the quantitative variables was assessed using the Shapiro-Wilk test. Non-parametric statistics were used to compare the levels of repeated measurements and *post hoc* non-parametric analysis was applied for between-time-point comparisons. All analyses were performed using Statistical Software, version 10.0.A (Stat soft) and a *P*-value <.05 was considered statistically significant.

3. Results

The salivary Ig A level correlated with serum IgA level at diagnosis of ALL. The Spearman correlation rank for the relationship was $r=0.28$ and $P=.031$ (Fig. 2).

During the examined period, the level of salivary IgA dropped from 10.7+/-4.8 to 9.6+/-6.4 between days 1 and 8, and to 5.7+/-3.9 (ng/mL) on day 22 ($P=.04$); (Table 2). This was even more pronounced when the level of salivary immunoglobulin A was normalized with the level of salivary total protein ratio, as the numbers decreased from 6,6+/- 3,2 on the first day, to 3,8+/-1,2 (ng/mg) on the last day of the study ($P=.02$); (Table 2).

Table 1

The characteristics of the study group.

		ALL-IC BFM	
Age at diagnosis median (yr, min-max)		10.16	(4.08–17)
Sex	F	11	45.83%
	M	13	54.16%
Risk group	SR	2	8.33%
	IR	16	66.66%
	HR	6	25%
	1	17	70.83%
CNS involvement	2	7	29.16%
	3	0	0
	T	7	29.16%
Immunophenotype	pre-B	17	70.83%
	Mucositis	present	4
Time at mucositis presentation	days of treatment	16+/-6	

CNS = central nervous system; HR = high risk group; IR = intermediate; SR = standard risk group.

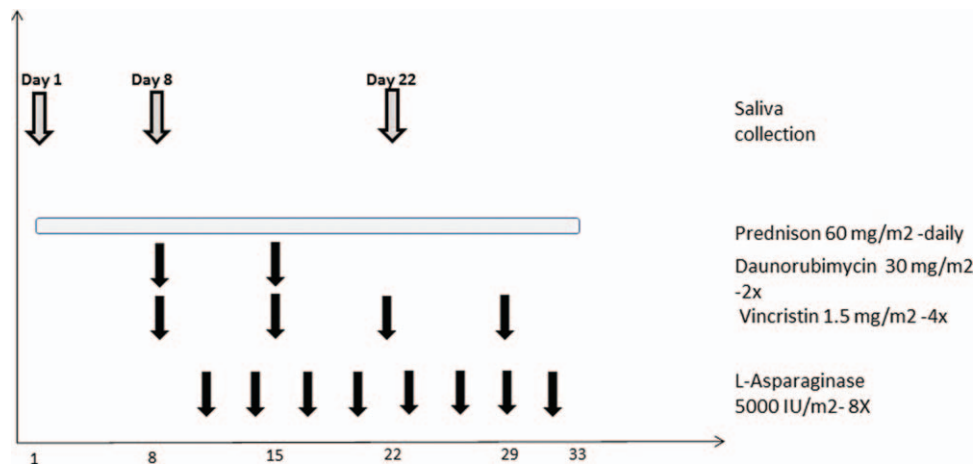


Figure 1. Remission induction treatment regimen (the first 33 days of therapy) for childhood acute lymphoblastic leukaemia according to the ALL-IC BFM 2009 protocol with denoted time-points when saliva was collected.

In saliva, the significant reduction of immunoglobulin A level was not associated with any changes in total protein, which remained constant during the first 22 days of the therapy. In blood, the total protein content dropped from 6.2 ± 0.4 to 5.1 ± 0.3 g/dL between days 1 and 22 ($P = .01$). The level of serum IgA also decreased, but not significantly.

A correlation between lymphocyte count and serum IgA level was observed ($r = 0.54$; $p < .03$); however, no correlation was found between lymphocyte count and salivary IgA level ($r = 0.06$; $P = .33$); (Fig. 3A and B). Total lymphocyte count (per microliter) decreased considerably between day 1 and day 8, and day 22 (2.12 ± 0.8 vs 0.41 ± 0.1 vs 1.08 ± 0.5 ; $P < .002$). More detailed data is given in Table 2.

No cases of mucositis were noted before day 8 of treatment; however, 4 children were found to be suffering from oral mucosal inflammation until day 22. Three cases of mucositis were graded as 1 (mild form) according to the common terminology criteria for adverse events criteria while 1 case was graded as 3 (severe). One of 3 patients with mucositis graded as 1 was also found to

display candidiasis of the oral mucosa. The level of salivary IgA at leukaemia diagnosis were similar among patients who later presented mucositis and who did not (10.8 ± 6.1 vs 10.6 ± 3.9 ng/mL, respectively; $P = .63$). During the treatment, the levels of salivary IgA were lower among 4 children suffering for mucositis than among children without mucosal lesions (at day 8: 9.1 ± 7.4 ng/mL vs 9.7 ± 5.4 ng/mL and $P = .31$, at day 22: 5.1 ± 4.9 ng/mL vs 5.7 ± 2.8 ng/mL and $P = .19$).

4. Discussion

The agents used in the treatment of lymphoblastic leukaemia according to the ALL-IC BFM 2009 protocol are highly cytotoxic to cancer cells. The initial stage of the treatment used prednisone, an anti-inflammatory synthetic glucocorticoid. This displays also ability to initiate apoptosis in sensitive tumour cell populations in leukemia and lymphoma.^[14] Typical steroid-related complications can be observed following prednisone use, but in general, no oral problems should be detected. This was confirmed by the fact that none of our patients suffered from acute oral inflammation in the initial stage of therapy. Between days 1 and 8, no significant changes in levels of salivary or serum IgA were noted. Also, no significant changes in total protein level were observed in both fluids.

The second phase of treatment saw the introduction of other cytotoxic drugs including daunorubicin, vincristine and L-asparaginase. This chemotherapeutics originate from different chemical groups with different modes of action; however, all are known to influence the state of oral health in cancer patients. Daunorubicin is known as a very toxic antibiotic, anthracycline topoisomerase inhibitor which prevents DNA replication or repair, RNA and protein synthesis. Due to its high cardiotoxicity, its use is now limited to acute leukaemia and other neoplasms. The side effects of daunorubicin are mostly associated with its cytotoxic activity, 1 of which may be stomatitis.^[15]

Mouth sores and hair loss might be also present after usage of vincristine, an alkaloid isolated from plants, which may inhibit cell mitosis, cellular respiration, nucleic acid and lipid biosynthesis. The toxicity of vincristine often results in peripheral neuropathy, the most severe complication of its use in cancer patients.^[16,17] The last agent, L- asparaginase, disturbs the

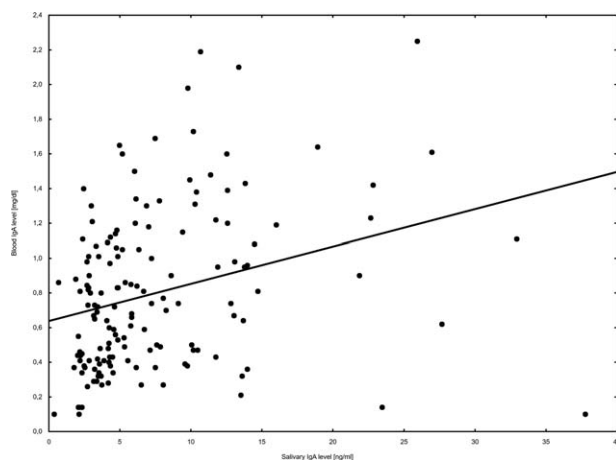


Figure 2. The relationship between levels of immunoglobulin A in blood and saliva, independently of the time point measured ($r = 0.28$; CI=95% and $P = .031$).

Table 2
Changes in salivary and serum proteins during remission induction treatment of ALL children.

Measurement	Day 1	Day 8	Day 22	P-level
	Mean +/- SD	Mean +/- SD	Mean +/- SD	
Salivary IgA level (ng/mL)	10.7+/-4.8	9.6+/-6.4	5.7+/-3.9	.04
Salivary protein (mg/mL)	1.6+/-0.6	1.5+/-0.8	1.5+/-0.9	.68
Salivary IgA/salivary total protein ratio (ng/mg)	6,6+/-3,2	6,4 +/-2,1	3,8 +/-1,2	.02
Serum IgA (mg/dL)	96.1+/-41.2	86.3+/-62.5	79.4+/-54.2	0.2
Serum total protein (g/dL)	6.2+/-0.4	6.3+/-0.8	5.1+/-0.3	.001
Blood total lymphocyte (per microliter)	2.12 +/-0.8	0.41+/-0.1	1.08+/-0.5	.002

SD = standard deviation.

biosynthesis of an essential amino acid, asparagine, resulting in cell death due to an inability to manufacture proteins.^[18] This happens not only to cancer cells, but also to healthy cells, and may lead to many health complications, 1 of which might be mouth sores.

In the present study, the level of total proteins in blood decreased significantly between days 8 and 22. In contrast to blood, the level of total protein in saliva remained almost constant until day 22. This fact may indicate that at the beginning of therapy, some compensating mechanisms in saliva might be present to neutralize the loss of proteins. It has been already proven that the salivary proteome changes after allogeneic hematopoietic stem cell transplantation,^[19] but the levels of a number of salivary proteins can decrease or increase. However, while the amount of total proteins in saliva remained unchanged, the level of salivary immunoglobulin A decreased. In general, immunoglobulin A is produced by activated B-lymphocytes at the surfaces of exocrine glands and all mucous membranes. IgA enters the circulatory system as a monomeric form (serum IgA), with a half-life of about 9 days; these monomers can bind with a J chain through their carboxyl ends to form dimeric IgA, the most commonly-occurring form of IgA in the organism.

In epithelial cells, dimeric molecules are enzymatically transformed to secretory IgA, transported by transcytosis and released to the mucosal site and fluids. These highly-sensitive mechanisms are regulated by the cytoskeleton, several Rab GT Pass and other signalling cascades.^[5] As the levels of Ig A in saliva and serum exist in a constant balance, the occurrence of a greater drop in saliva than in serum indicates that immunoglobulin transforma-

tion or transport in the cell must have been disturbed. Our present findings also showed that the general lymphocyte count dropped over the course of the 22-day observation; a fall that strictly correlated with a fall in the levels of serum immunoglobulins produced by the cells. However, the fall in blood lymphoblast count did not correlate with the fluctuation of IgA levels in saliva. This supports our new hypothesis that the transformation of immunoglobulins from serum to secretory forms is impaired by the chemotherapeutic used during oncotherapy, and this should be the matter of more advanced investigations in the future.

It has been found that the level of IgA in saliva in healthy patients varies from 4 to 40 microgram/mL,^[1,19,20] while the level of total protein in saliva was assessed as 0.9 to 1.65 mg/mL.^[21,22] In general, the level of immunoglobulin in saliva, as well as those of other constituents, depends on many factors, including age, sex, medication intake, stress, physical activity or even diet.^[1-4] The state might also be changed during head and neck radiotherapy, which has been widely discussed in the literature.^[23] Hypo salivation has previously been determined in children with Hodgkin disease immediately after radiotherapy and 1 month later, while the level of immunoglobulin in saliva remained elevated for the next 2 months.^[24]

However, most contemporary studies have found the level of salivary IgA to be depressed in cancer patients. In patients with oral squamous cell carcinoma, the concentration of IgA was approximately 45% lower than in healthy controls.^[25] Such decreases were also observed in cancer patients several months after autologous bone transplantation and up to 5 years after the treatment of Hodgkin lymphoma.^[4,26] Moreover, reduced levels

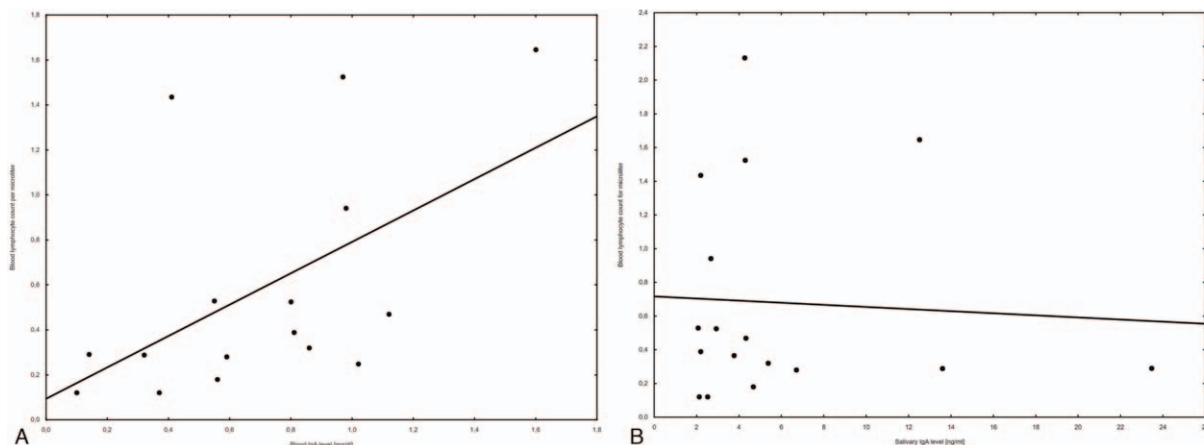


Figure 3. a, b The significant correlation between lymphocyte count and serum IgA level ($r=0.54$; $P<.03$); (panel a), and the lack of correlation between lymphocyte count and salivary IgA level ($r=-0.06$; $P=.33$); (panel b).

of salivary Ig A have been associated with increased mortality in both cancer and control patients.^[27] Significantly greater reductions in salivary IgA level have also been found in children suffering from mucositis.^[10] We observed only slightly lower levels of salivary IgA concentrations among ALL patients who presented mucositis, which is possible related to small number of patients with mucosal damage found in the study group.

To increase the statistical power, we recruited only ALL patients, which formed a relatively homogenous group and these individuals were unified by being treated with the same regimen. However, further studies are needed to draw more solid conclusions, especially concerning the link between mucositis and salivary IgA level. We are aware that for this goal our study was underpowered. Additionally, an integrated mucosal immunity is more complex and does not rely only on secretory immunoglobulins. Therefore, another limitation of the study is that we focus only on 1 component of mucosal immune system. However, for the first time our results have shown a link between intensive anti-cancer chemotherapy and salivary immunoglobulins.

5. Conclusions

An intensive anti-cancer treatment of paediatric patients with ALL may affect the level of salivary immunoglobulin A. Since salivary immunoglobulins are important for integration of mucosal immunity, 1 may speculate that observed decrease in IgA level may escalate the risk of occurrence of acute mucosal complications; however this statement needs further studies.

Author contributions

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