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Determination of Glucocorticoid Receptor α and β mRNA Expression Levels on Pediatric ALL Patients and Evaluation of Relationship Between α/β Ratio and Glucocorticoid Resistance

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Abstract

Glucocorticoids have been used in chemotherapy regimens of leukemia for their lymphocytic effect. Glucocorticoid resistances (GC) remain as an important problem and the exact mechanism has not been elucidated. Many studies have been reported describing the relation between response and Glucocorticoid Receptors α/β (GR α/β) expression in childhood leukemia. In present study aimed to determine expression levels of glucocorticoid receptors and to evaluate the relationship with clinical parameters and development of glucocorticoid resistance in children with acute leukemia. To test this hypothesis, GRα/β messenger-RiboNucleic Acid (m-RNA) expression levels were determined in 13 children wtih acute leukemia patients. This study 'results reported that GRβ expression levels were high at leukemia. The results in this article seem to be the first screening results of the GRα/β m-RNA expression levels studied by quantitative Real Time Polymerase Chain Reaction (qRT- PCR) in Turkish acute leukemia patients. The cause of GC resistance in childhood leukemia is largely unknown. The clinical glucocorticoid resistance positivity and the GRα/β ratio may be effective in leading leukemia in glucocorticoid resistance.

1. Introduction

Glucocorticoids have been used in chemotherapy regimens of different type of leukemia for their lympholytic effect mainly by activating intrinsic apoptotic signaling pathways. Glucocorticoids have two major GR; human (h) GRα and hGRβ. These two variants have expressed by alternative splicing of GR gene. Figure 1 shows alternative splicing regions and structure of the glucocorticoid receptor gene. It has been shown that GRβ has a dominant negative effect on transcriptional activity of GRα [1-3].
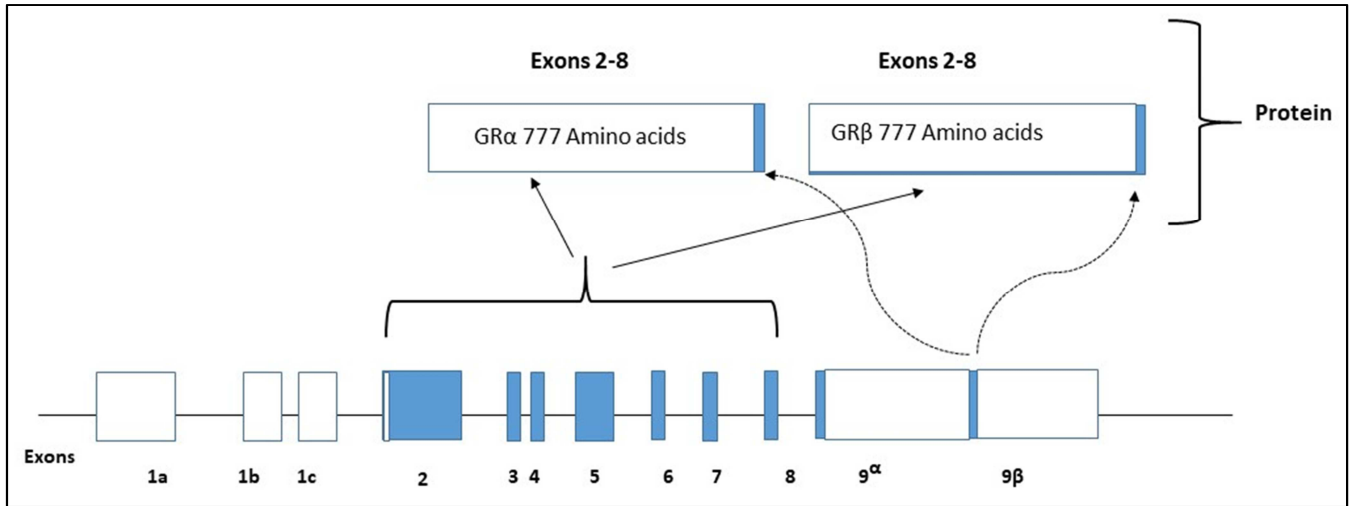
Figure 1. Structure of the glucocorticoid receptor gene.

Glucocorticoid resistance remains as an important problem even in contemporary chemotherapy protocols. Although several studies have been done to clarify the reason for glucocorticoid resistance, the exact mechanism has not been elucidated to date. Berlin-Frankfurt-Münster (BFM) protocol was used for the treatment of acute lymphoblastic leukemia (ALL). Early response to glucocorticoid treatment is accepted as important prognostic criteria for patients. Glucocorticoid response is determined by blast count of the 8th day of the therapy (blast count $>1000\rightarrow$glucocorticoid resistance). It has been shown that glucocorticoid resistance in patients with leukemia is related with increased expression of GRβ and decreased GRα/β ratio [5-7]. However, these studies had been performed mostly on cancer cell lines and the number of studies using the patient's sample are scarce.

There a few study which investigates GRα/β in childhood leukemia patients. Therefore in present study, to investigated GRα and GRβ mRNA expression levels in children with acute leukemia patients by using qRT-PCR and to assess the ration of expression levels of GRα/β [4-7]. In this study's aim was to determine GRα and GRβ mRNA expression levels in pediatric ALL patients and to evaluate the relationship between GRα/β ratio and development of glucocorticoid resistance.

2. Material and Methods

Thirteen pediatric ALL patients aged between 1-15 and two pediatric controls were recruited. The blood samples were drawn at diagnosis and the 8th day of the glucocorticoid treatment. The RNAs were isolated by using Biostic Stabilized Blood RNA isolation kit. Quantity and quality of RNAs were determined by Nanodrop. Genes Expression analysis was performed by qRT–PCR technique and Sybr Green kit on Light Cycler 480II (Roche). Results were analyzed by Basic Relative Quantification software ($2^{-\Delta\Delta Ct}$-metod) and GRα/β expression ratios were calculated. Table 1 summaries demographic characteristics of patients. The study is carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The Ankara University, School of Medicine Ethics Committee approved the study protocol (project No. 03-107-13/2013) and informed consent was provided by the patients' parents.

Table 1. Demographic Characteristics, GRα/β Ratio and Glucocorticoid Resistance of Children with Leukemia.

Patients	Gender	Code	Diagnose	Glucocorticoid Resistance
M.K.U	Male	P1*	**Pre B-ALL	Negative
S.T	Male	P2	Pre B-ALL	Negative
M.B.E	Female	P3	Pre B-ALL	Negative
S.K	Female	P4	Pre B-ALL	Negative
A.N.A	Female	P5	Pre B-ALL	Positive
M.Y	Female	P6	Pre B-ALL	Negative
E.C	Male	P7	Pre B-ALL	Positive
M.E.C	Male	P8	Pre B-ALL	Negative
S.N.G	Female	P9	Pre B-ALL	Negative
M.I	Female	P10	***T-ALL	Negative
B.O	Male	P11	T-ALL	Positive
S.A	Female	P12	T-ALL	Negative
S.K	Female	P13	T-ALL	Negative

*P: Patient
**Pre B-ALL: Precursor B cell Acute Lymphoblastic Leukemia
***T-ALL: T cell Acute Lymphoblastic Leukemia

3. Results

In this study, the result showed that there were variable expression patterns in GRα expression in pre-B-ALL samples when compared to controls. Unlike GRα, increases of GRβ expressions were detected in the entire patient samples. Hence, GRα/β expression ratios for these patients (except for P9) were significantly low. The ratios were showed in Figure 2.
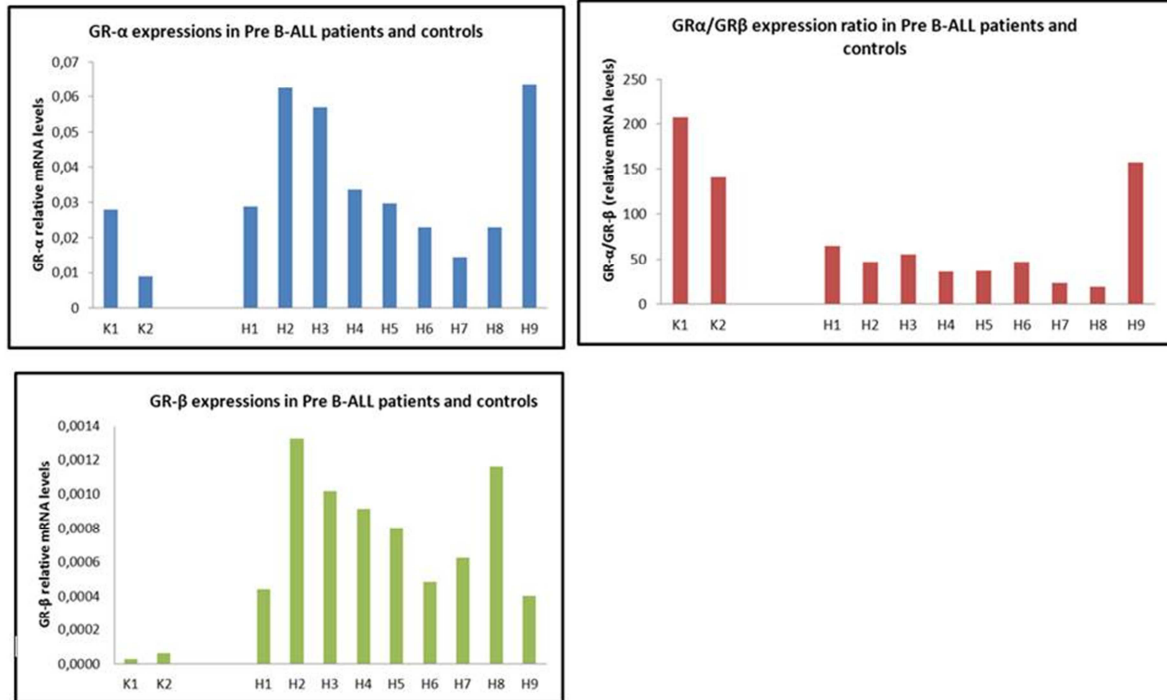

Figure 2. GR-α, GR-β mRNA levels and GRα/β ratios of Pre B-ALL patients and controls.

In T-ALL patient group GRα expressions were low on the ¾ of patients but GRβ expressions were high the entire patient samples compare to controls. GRα/β expression ratio was low in the T-ALL group. The ratios were showed in Figure 3.
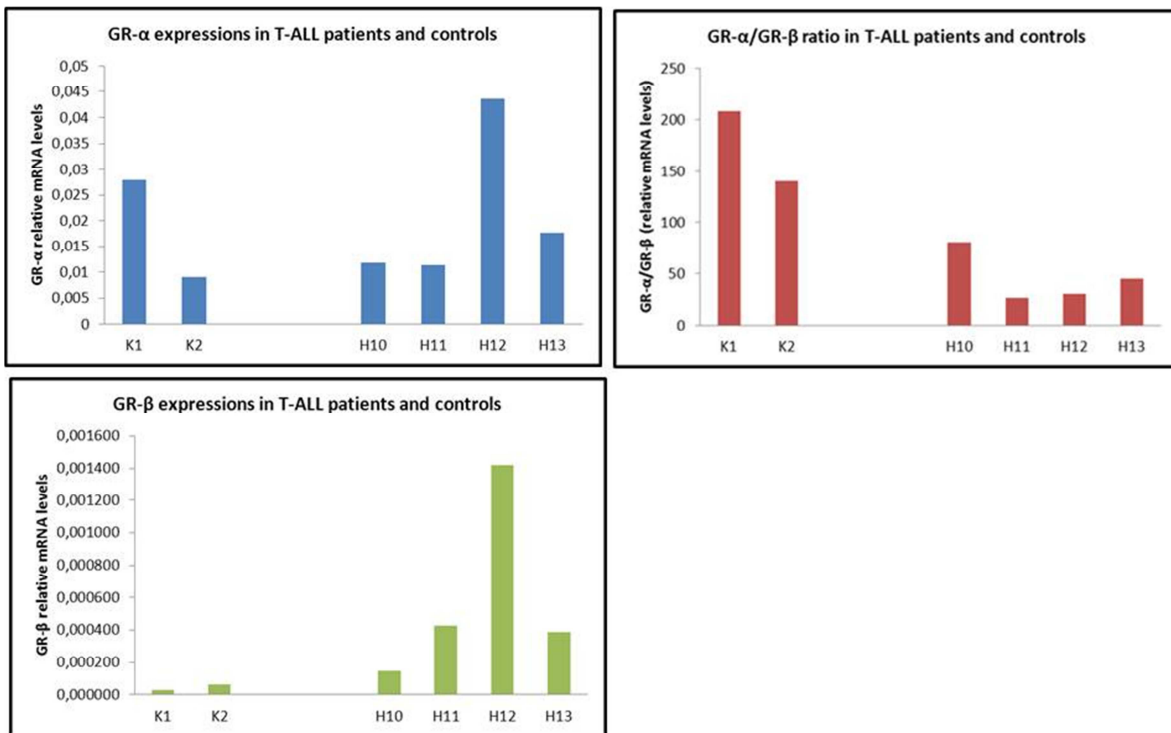

Figure 3. GR-α, GR-β mRNA levels and GRα/β ratios of T-ALL patients and controls.

This study reported that GRβ expression levels were high at both of the leukemia. This causes striking decrease in the GRα/β expression ratio. The results of the analyses and clinical glucocorticoid resistance are shown in Table 1. Pre-B-ALL study group, except one patient, showed low GRα/β ratio but only two patients who had low ratio displayed clinical glucocorticoid resistance. The Table 1 summaries glucocorticoid Resistances. T-ALL study group presented low GRα/β ratio and one patient from this group displayed clinical glucocorticoid resistance. The patient drew attention because it has glucocorticoid resistance as well as the lowest GRα/β ratio. Therefore, these results argue that it could be an association between low GRα/β ratio and glucocorticoid resistance in T-ALL but not pre-B-ALL. Data obtained from Pre B-ALL patients suggest that other factors, such as alternatively spliced sub-groups of glucocorticoid receptors and epigenetic factors might be play role in the development of glucocorticoid resistance.

4. Discussion

Acute leukemia is a heterogeneous disorder of hematopoietic stem cells, characterized by multiple genetic events that have an impact on proliferation and differentiation. Some of the genetic and epigenetic alterations play a major role in leukemogenesis; gene mutations, deletions, translocations, and DNA methylation and resistance of therapy. Glucocorticoids have been widely used as immunosuppressive and anti-inflammatory agents in the treatment of numerous autoimmune and inflammatory diseases. Glucocorticoids also play an important role in anti-cancer therapy. Activation of the immune system is described as an important treatment strategy against cancer. However, glucocorticoids inhibe the immune system and many studies have shown that immunosuppression can exacerbate the metastatic process and accelerate tumor growth. A number of studies have been conducted to elucidate the resistance and resistance of glucocorticoid therapy to leukemia patients [8-10].

There are studies reporting that glucocorticoid resistance in leukemic patients is associated with increased GRβ expression and decreased GRα / β ratio. Longui et al reported that low GRα, high GRβ and GRα /β ratio were detected in T-ALL patients [6]. Furtermore, levels of GRβ mRNA were found to be high and reduced activity of GRα was detected in asthma patients with glucocorticoid insensitive [7]. The present study showed that the results of T-ALL subgroup supported the hypothesis that GRα/β expression ratio could be related with glucocorticoid resistance seen during chemotherapy.

5. Conclusion

In conclusion, the low expression levels of GRb mRNA

plays as a crucial inhibitör of GRα activity in childhood leukemia. Data obtained from Pre B-ALL patients suggest that other factors, such as alternatively spliced sub-groups of glucocorticoid receptors and epigenetic factors might play a role in the development of glucocorticoid resistance.

Conflict of Interest Statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/ or affiliations relevant to the subject matter or materials included.

References

[1] Kofler R. (2000). The molecular basis of glucocorticoid-induced apoptosis of lymphoblastic leukemia cells. *Histochem Cell Biol,* 114: 1-4.

[2] Distelhorst CW. (2002). Recent insights into the mechanism of glucocorticosteroid-induced apoptosis. *Cell Death Differ,* 9 (1): 6-19.

[3] Hollenberg SM., Weinberger C., Ong ES., Cerelli G., Oro A., Lebo R., Thompson EB., Rosenfeld MG., Evans RM.(1985). Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature*, 318: 635-41.

[4] Bamberger CM., Bamberger AM., de Castro M., Chrousos GP. (1995). Glucocorticoid receptor beta, a potential endogenous inhibitor of glucocorticoid action in humans. *J Clin Invest*, 95 (6): 2435-41.

[5] Haarman EG., Kaspers GJ., Pieters R., Rottier MM., Veerman AJ. (2004). Glucocorticoid receptor alpha, beta and gamma expression vs in vitro glucocorticod resistance in childhood leukemia. *Leukemia*, 18 (3): 530-7.

[6] Longui CA., Vottero A., Adamson PC., Cole DE., Kino T., Monte O., Chrousos GP. (2006). Low glucocorticoid receptor alpha/beta ratio in T-cell lymphoblastic leukemia. *Horm Metab Res,* 32 (10): 401-6.

[7] Goleva E., Li LB., Eves PT., Strand MJ., Martin RJ., Leung DY. (2006). Increased glucocorticoid receptor beta alters steroid response in glucocorticoid-insensitive asthma. *Am J Respir Crit Care Med*, 173 (6): 607-16.

[8] Moharram SA., Shah K., Kazi JU. (2017). T-cell Acute Lymphoblastic Leukemia Cells Display Activation of Different Survival Pathways. *J Cancer*, 6; 8 (19): 4124.

[9] Miller AL., Geng C., Golovko G., Sharma M., Schwartz JR., Yan J., Sowers L., Widger WR., Fofanov Y, Vedeckis WV., Thompson EB.(2014). Epigenetic alteration by DNA-demethylating treatment restores apoptotic response to glucocorticoids indexamethasone-resistant human malignant lymphoid cells. *Cancer Cell Int*, 23; 14: 35.

[10] Haarman EG., Kaspers GJ., Pieters R., Rottier MM., Veerman AJ. (2004). Glucocorticoid receptor alpha, beta and gamma expression vs in vitro glucocorticod resistance in childhood leukemia. *Leukemia,* 18 (3): 530-7.