University of Thi-Qar Journal of Science (UTJsci)
E-ISSN: 2709-0256, P-ISSN: 1991-8690, Vol. 11, No. 1, Jun. 2024

Investigation of the Serotonin, Cortisol Hormones and some Biochemical Parameters in Patients with Beta Thalassemia in Thi-Qar Governorate /Iraq

Noor R. Ali *[1a], Leila Sadeghi[2b], Gholamreza Dehghan [2c]
[1]*Department of Chemistry, College of Science, University of Thi-Qar, Thi-Qar, 64001, Iraq.*
[2]*Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz , Tabriz, Iran.*
[b]E-mail: l.sadeghi@tabrizu.ac.ir , [c]E-mail: Dehghan2001d@yahoo.com

[a]*Corresponding author: noor.r.ali@sci.utq.edu.iq

Received: 2024-03-04, Revised: 2024-03-23, Accepted: 2024-03-30, Published: 2024-06-20

***Abstract*—** Beta thalassemia (β-thalassemia) is the most severe kind of β-thalassemia hereditary disease that makes blood transfusion dependent. Patients with β-thalassemia disease experience many physiological abnormalities, including oxidative stress, iron overload, and aggregated β-globin proteins. These abnormalities may also lead to premature RBC mortality. In the present study, 100 patients with β-thalassemia major with an average age of 16.30 ± 6.67 years old and 30 healthy controls with an average age of 14.63 ± 4.17 years old were included in this study. The serum levels of urea, creatinine, serotonin, and cortisol were investigated in the patient and control groups. The results showed a marked increase in the urea levels among the patients compared to the control group (from 14.63 ± 6.05 mg/dl in the control group to 24.98 ± 11.35 mg/dl in patient group) with P <0.001, rendering this change highly statistically significant. While there was a noticeable increase in creatinine levels between patients and controls (from 0.387 ± 0.199 mg/dl in the control group to 0.466 ± 0.208 mg/dl in the patient group. Statistical analysis revealed this change with non-significance of P = 0.066. Furthermore, the obtained results indicated that the serum level of serotonin decreased in patient group (from 393.46 ± 192.42 ng/ml in control group to 385.63 ± 167.70 ng/ml in patient group) with P=0.884, while β-thalassemia major subjects showed a significantly higher level of cortisol compared to the healthy control group (from 94.97 ± 67.56 ng/ml in control group to 304.37 ± 173.81 ng/ml in patient group) with P < 0.001. These findings showed an inverse correlation between the serum levels of serotonin, cortisol, and β-thalassemia major diseases.

***Keywords*– β-thalassemia, Serotonin, Cortisol, Urea**

I. Introduction

A thalassemia is a diverse group of hereditary disorders that are transmitted via autosomal recessive inheritance. They are characterized by a partial or total suppression of the rate at which the two chains of adult hemoglobin (Hb A) are synthesized, resulting in a lower rate of synthesis than in normal hemoglobin production [1]. The hemoglobin polypeptide chains alpha and beta are encoded by the human globulin genes which are located on chromosomes 16 and 11, respectively. Alpha/beta-thalassemia (α-/β-thalassemia) is the result of deficient or absent synthesis of α/β globin chains, leading to excess β/α globin chains. Hemoglobinopathies in thalassemia are caused by genetic defects in the related genes resulting in reduced or no production of one globulin chain [2].

Hemolysis, or the breakdown of red blood cells in the bone marrow and peripheral circulation, results from an imbalance in the synthesis of globin chains in thalassemia [3]. Aplastic anemia, myelodysplastic syndrome, sickle cell disease, glucose-6-phosphate dehydrogenase (G6PD) deficiency, spherocytosis, and different types of β-thalassemia are all associated with lipid troubles [4-6].

In thalassemia patients, various lipoproteins, cholesterol, and triglycerides exhibit significant oxidative modification due to free radical injury, which could indicate a pathogenesis-related event [7]. Patients with excess iron have elevated levels of free radical generation. Heart-related iron overload and hypertriglyceridemia are the main causes of many cardiovascular problems that affect individuals with thalassemia. The three basic β-thalassemia phenotypes tend to be identified based on the clinical presentation, with the understanding that certain phenotypes are frequently, but not always, associated with particular chain genetic profiles. The β-thalassemia minor, or β-thalassemia trait, is characterized by a heterozygous inheritance of β-thalassemia mutation, resulting in borderline asymptomatic anemia with hypochromic and microcytosis [8]. It has been reported that thalassemia has a significant effect on the endocrine system function and can be investigated through its genetic basis. The main objective of the present study was to evaluate the serum levels of cortisol and serotonin in patients with β-thalassemia in the province of Thi-Qar, Iraq.

II. Patients and Methods

A. Collection of samples

The current study included 30 healthy control individuals and 100 patients with thalassemia major. The serum samples were collected from The Center of the Genetic Blood Diseases in Thi-Qar province in Iraq country and stored at a temperature of -20°C for biochemical analysis. A total of 10 mL of venous blood was collected from the control subjects and patients for the study, and the process was carried out as follows: a volume of 2.5 mL of venous blood was withdrawn from the patient and placed in special Gel tube tests. In

https://doi.org/10.32792/utq/utjsci/v11i1.1186

addition, the serum levels of serotonin and cortisol were analyzed using commercial ELISA kits, including the Serotonin/5-Hydroxytryptamine ELISA Kit, the Human Cortisol ELISA Kit, from Ela Science/ China., Urea, and creatinine Assay Kit Abcam.

A. Exclusion criteria

Excluded patients from our study were patients with a history of dementia in their family, kidney disease, thyroid disorders, acute coronary syndromes, Alzheimer's disease, diabetes, pregnancy, anti-obesity medication users, and comorbid conditions like HIV, COVID-19, cancer, and chronic obstructive pulmonary disease.

B. Statistical analysis

All of the experiments were repeated at least three times independently, and the data were reported as the mean ± standard deviation (SD) using the Graph Pad in Stat version 10.0.2 program (Graph Pad Software, San Diego, CA). By computing values with the Student's *t*-test at a 5 % significance level, statistical significance was set at $p < 0.05$.

III. Results and Discussion

A. Demographic characteristics of patients with thalassemia major and healthy control subjects

The demographic and some other study-relevant characteristics of the patients and control participants included in the present study are listed in Table 1. According to this table, a total of 130 subjects participated in this work, in which both patients and control groups were comprised of 64 females and 66 males. The results indicated that there was no significant difference in the mean age of patients and control individuals (P = 0.199). The mean age of the patients was 16.30 ± 6.67 years, and that of the control groups was 14.63 ± 4.17 years. Furthermore, findings showed no discernible difference between the age group frequency distribution of the control participants and patients (P = 0.154).

TABLE I. Demographic characteristics of individuals with thalassemia major and healthy controls.

Variable	Patients ± SD	Control ± SD	*p-value*
Age (years)	16.30 ± 6.67	14.63 ± 4.17	0.199 ¥ NS
Range	4– 32	8 – 22	
Sex, n (%)			
Male	**52 (52.0 %)**	**14 (46.7 %)**	**0.608 ¥ NS**
Female	**48 (48.0%)**	**16 (53.3%)**	

The variables SD, Y, and NS denote the standard deviation, and Chi-square test, and are not significant at P > 0.05, respectively.

The sex frequency distribution of the patients and control participants did not significantly differ (P = 0.608), with 52 (52.0%) males and 48 (48.0%) females in the patients' group and 14 (46.7%) males and 16 (53.3%) females in the control group. Based on these results, it can be concluded that in case-control studies, age, and sex bias can be neglected if there were u7m/\[no appreciable changes in the distribution of individuals in both groups.

B. Various biochemical marker levels in healthy controls and thalassemia major patients

Blood urea level or urea nitrogen is a chemical compound that is produced in the body as a byproduct of protein breakdown. The liver typically synthesizes urea during the process of protein metabolism, and it is transported via the bloodstream to the kidneys for excretion. Measuring blood urea levels can be useful in the diagnosis and monitoring of certain conditions and diseases. The levels of blood urea were 24.98 ± 11.35 mg/dl and 14.63 ± 6.05 mg/dl in the patient and healthy groups, respectively. These findings revealed that the level of blood urea was higher in the patient group compared to the healthy control subject, and the difference was very significant (P <0.001). Figure 1 compares the serum levels of blood urea in patients with control subjects.

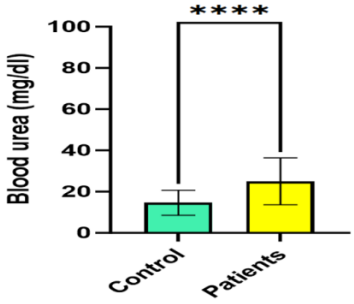

Fig. 1. Mean levels of blood urea of patients and control subjects. All data were expressed as mean ± SD. **** was indicated on the column graph in significant changes (P<0.0001).

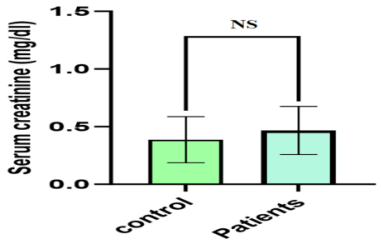

Fig .2. Serum levels of creatinine in patients and control subjects. All data were expressed as mean ± SD. NS was indicated on the column graph in nonsignificant changes (P> 0.05).

Creatinine is a chemical compound that is produced in the muscles of the body and naturally eliminated by the kidneys. Measuring the level of creatinine in the blood can help evaluate kidney function and diagnose certain kidney-related conditions and states. According to the results, the serum levels of creatinine in the patients and the healthy control subjects were 0.466 ± 0.208 mg/dl and 0.387 ± 0.199 mg/dl, respectively. As shown in Figure 2, the patient group had a higher level of creatinine than the healthy control subject, but the difference was not statistically significant (P = 0.066).

C. Serum level of cortisol

Cortisol is a steroid hormone produced by the adrenal glands in the body. This hormone plays a crucial role in regulating growth, metabolism, immune system function,

and stress response. In patients with β-thalassemia, the deficiency in hemoglobin production and disturbances in iron metabolism can potentially affect cortisol levels in the body. Factors such as increased stress, recurrent infections, and immune dysfunction can contribute to elevated cortisol levels. Additionally, in some cases, patients with β-thalassemia may experience changes in cortisol levels due to the use of synthetic blood transfusions and steroids. Measuring cortisol levels in patients with β-thalassemia can serve as an auxiliary marker for evaluating stress levels, adrenal gland function, and stress response [9]. According to Figure 3, the mean serum cortisol levels in the patient groups were 304.37 ± 173.81 ng/ml, while the healthy control group had a mean level of 94.97 ± 67.56 ng/ml. These results showed that the serum level of cortisol in patients was substantially higher than that in the healthy control group (P < 0.001).

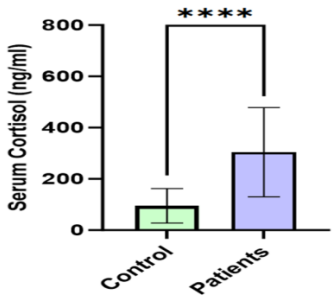

Fig. 3. Mean serum cortisol level of patients and healthy controls. All data were expressed as mean ± SD. **** was indicated on the column graph in significant changes (P<0.0001).

D. Serum serotonin/5-HT level in patients and healthy control

Serotonin is a chemical messenger that is produced in the central nervous system and peripheral tissues of the body. It plays a crucial role in regulating mood, sleep-wake cycles, cell growth and division, and gastrointestinal function. In patients with beta-thalassemia, disruptions such as decreased hemoglobin production and iron overload can potentially lead to changes in serotonin levels in the body. Additionally, patients with β-thalassemia may experience alterations in serotonin levels due to the use of synthetic blood transfusions and medications such as deferoxamine. The mean levels of serum serotonin/5-HT were 385.63 ± 167.70 ng/ml and 393.46 ± 192.42 ng/ml in the patient group and healthy control, respectively. These results indicated that, compared to the healthy control group, the serum level of serotonin/5-HT was slightly and not significantly lower in the patient group (P= 0.884) (Figure 4).

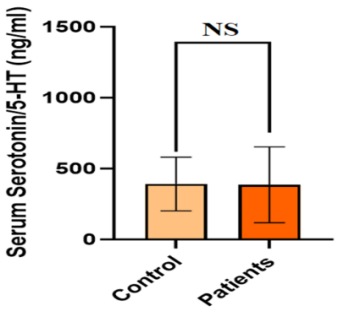

Fig. 4. Mean serum serotonin/5-HT level of patients and healthy controls. All data were expressed as mean ± SD. NS was indicated on the column graph in nonsignificant changes (P> 0.05).

IV. DISCUSSION

Based on certain studies, a marker of decreased kidney function can be used to determine whether β-thalassemia major is associated with impaired renal function. Biomarkers are quantifiable biological components that distinguish between damage and normal function. Acute kidney damage can be detected early using several unique urine biomarkers. In a variety of renal lesions, tubular cells create and release these indicators as quantifiable proteins. Since renal problems can be linked to numerous short- and long-term repercussions, assessments of kidney functions in β-thalassemia patients are typically performed in clinics. Early detection of kidney impairment may be crucial for providing the best care and improving patient outcomes. Therefore, it is crucial to identify an early and trustworthy biomarker of kidney involvement in thalassemia. The results of our study demonstrated a substantial positive correlation between the number of blood transfusions and urea levels in both blood and saliva. For the rest of their lives, patients with β-thalassemia major require blood transfusions every two to five weeks to prevent anemia and maintain hemoglobin levels between 9 and 10 g/dl. The main causes of kidney failure in patients with β-thalassemia major are excessive erythrocyte hemolysis from erythropoiesis and iron deposits from frequent blood transfusions. The body's iron load is increased by red blood cells from blood transfusions by 1 mg/ml [10-14].

The serum level of urea in the patient group in our current investigation was greater than the healthy control group, and this difference was statistically significant (P< 0.001). Furthermore, serum creatinine (Cr) concentration in the patient group (0.466 ± 0.208 mg/dl) was higher than in the healthy control group (0.387 ± 0.199 mg/dl), although the difference was not statistically significant (P = 0.066). Numerous studies are in good agreement with this investigation. Recent studies found that serum urea levels were considerably greater in Cr-β-thalassemia patients than in controls, but no significant difference was observed [15, 16]. However, different authors discovered normal serum Cr in β-thalassemia patients [17]. According to the previous study, patients with thalassemia have elevated serum Cr levels [18]. Furthermore, other studies could not discover a significant difference between patient and control levels of serum urea, Cr, or serum and urine electrolytes, except for a higher urinary protein/Cr ratio in thalassemia patients [19].

The lower body mass index for β-thalassemia patients typically experiences growth retardation and decreased muscle mass may be responsible for the significant decrease in the mean serum creatinine level. According to the results of recent studies, the main prospective causes of morbidity and mortality in β-thalassemia patients were liver disease, cardiac disorders, diabetes, infection, and cancer due to long-term complications [20, 21]. Due to socioeconomic inequities in our nation, the majority of β-thalassemia patients do not receive iron chelating therapy regularly. As a result, β-thalassemia patients frequently received blood transfusion therapy, and some of them, especially in rural areas, received insufficient iron chelating agents. This study investigated and compared the levels of four factors in the blood serum to those of the control group. In this study, when we compared the level of serum cortisol in the healthy control group with the patient group the level of serum cortisol in the patient group was significantly higher ($P< 0.001$). Serum cortisol levels in patients under 10, those between 10 and 19 years old, and those over 20 years old were found to be 149.56 ±96.12 ng/ml, 245.55 ± 146.12 ng/ml, and 481.75 ±83.33 ng/ml, respectively. Patients in the older age groups had mean levels that were higher than the patients in the other groups, and the difference was highly significant ($P = 0.001$). Though slightly higher in the female groups, the mean levels in the male groups were not significantly different from the levels in the female groups. Studies reported that primary or secondary adrenal insufficiency is also possible when the cortisol-producing adrenal glands are injured and cannot create enough of the hormone. Aldosterone, an adrenal hormone, may also be deficient [22]. It was unclear what pathophysiological factors contributed to adrenal insufficiency in thalassemia major. Adrenocorticotropic hormone (ACTH) production may be inhibited by iron accumulation in the pituitary, which could result in secondary adrenal insufficiency. Iron toxicity may also directly impact the adrenal glands, resulting in primary adrenal insufficiency [23]. According to other studies, participated patients in their research had normal ACTH and cortisol levels, but the results indicated that the growth hormone and cortisol responses were insufficient [24]. Our findings indicated that the patient's cortisol levels increased, and it agrees with other studies. The study's findings also showed that patients with low baseline levels of cosyntropin had secondary adrenal insufficiency, which supported the use of the low-dose ACTH stimulation test rather than the high-dose test to look at the hypothalamic-pituitary-adrenal axis in these patients. It was found that the low-dose test could effectively find concealed secondary adrenal insufficiency in patients with a normal baseline cortisol level [25]. Serotonin (5-hydroxytryptamine, or 5-HT), a multifunction bioamine with significant signaling functions in several physiological pathways, is a mood regulator. Serotonin (5-HT) is a hormone that regulates many physiological processes as well as a major neurotransmitter in the central nervous system. It is mostly known to function in the regulation of mood, sleep, and anxiety [26]. The blood condition β-thalassemia major is inherited and persistent. Transfusions of blood are necessary for patients. Their situation might become quite challenging due to depression, anxiety, and stress. According to a study conducted in Egypt to determine the prevalence of anxiety and depression in people with thalassemia, 32.1% and 16.1% of patients, respectively, reported having clinical or borderline depression [26, 27]. According to a different study done in Palestine, the majority of thalassemia patients (78.5%) who have high ferritin levels also have moderate to severe depression (55.8% of the participants had severe depression, while 22.7% had moderate depression) [28]. According to another study, patients with significant depressive disorders had greater levels of iron in several brain regions, which was linked to the severity of their depression [29]. In our study, serum serotonin/5-HT levels were 393.46 ng/ml and 385.63 ng/ml, respectively. These results revealed that the patient group's serum levels were marginally not significantly lower than the healthy controls. Studies have demonstrated that individuals with β-thalassemia develop depression. The study of serotonin levels in patients with β-thalassemia major showed dramatically reduced serotonin levels in patients when compared with healthy controls [30]. In many chronic disorders, including β-thalassemia, depression is a common psychological problem [31].

V. CONCLUSION

Patients with Multiply transfused β-thalassemia are prone to metabolic and hormonal problems. According to recent studies, β-TI is not a benign condition as previously believed; instead, it is linked to a larger range of organ dysfunction and comorbidities, as well as increased morbidity. Therefore, in the present study the serum levels of cortisol and serotonin in β-thalassemia major patients were investigated. Our findings indicated that, compared to the control group, the serum levels of the cortisol and serotonin hormones in β-thalassemia major subjects increased and decreased, respectively. Therefore, the findings of this study demonstrated the need for cortisol and serotonin hormonal assessment in people with β-thalassemia major.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES

[1] D. Rund and E. Rachmilewitz, "β-Thalassemia," *New England Journal of Medicine*, vol. 353, no. 11, pp. 1135-1146, 2005.

[2] M. H. Steinberg, B. G. Forget, D. R. Higgs, and D. J. Weatherall, *Disorders of hemoglobin: genetics, pathophysiology, and clinical management*. Cambridge University Press, 2009.

[3] A. Kattamis, G. L. Forni, Y. Aydinok, and V. Viprakasit, "Changing patterns in the epidemiology of β-thalassemia," *European Journal of Haematology*, vol. 105, no. 6, pp. 692-703, 2020.

[4] F. B. Piel and D. J. Weatherall, "The α-thalassemias," *New England Journal of Medicine*, vol. 371, no. 20, pp. 1908-1916, 2014.

[5] E. Khandros, C. S. Thom, J. D'Souza, and M. J. Weiss, "Integrated protein quality-control pathways

regulate free α-globin in murine β-thalassemia," *Blood, The Journal of the American Society of Hematology*, vol. 119, no. 22, pp. 5265-5275, 2012.

[6] A. Premawardhena *et al.*, "A novel molecular basis for β thalassemia intermedia poses new questions about its pathophysiology," *Blood*, vol. 106, no. 9, pp. 3251-3255, 2005.

[7] A. Basak *et al.*, "Control of human hemoglobin switching by LIN28B-mediated regulation of BCL11A translation," *NatureGeneticss*, vol. 52, no. 2, pp. 138-145, 2020.

[8] M. Uda *et al.*, "Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of β-thalassemia," *Proceedings of the National Academy of Sciences*, vol. 105, no. 5, pp. 1620-1625, 2008.

[9] C. Hershko, "Pathogenesis and management of iron toxicity in thalassemia," *Annals of the New York Academy of Sciences*, vol. 1202, no. 1, pp. 1-9, 2010.

[10] F. Centis *et al.*, "The importance of erythroid expansion in determining the extent of apoptosis in erythroid precursors in patients with β-thalassemia major," *Blood, The Journal of the American Society of Hematology*, vol. 96, no. 10, pp. 3624-3629, 2000.

[11] J. Yuan *et al.*, "Accelerated programmed cell death (apoptosis) in erythroid," 2011.

[12] P. Pootrakul *et al.*, "A correlation of erythrokinetics, ineffective erythropoiesis, and erythroid precursor apoptosis in Thai patients with thalassemia: Presented in part at the XXI Congress of the International Society of Hematology, Sydney, Australia, May 1985, and the Annual Meeting of the American Society of Hematology, San Diego, CA, December 1997," *Blood, The Journal of the American Society of Hematology*, vol. 96, no. 7, pp. 2606-2612, 2000.

[13] D. Mandal *et al.*, "Caspase 3-mediated proteolysis of the N-terminal cytoplasmic domain of the human erythroid anion exchanger 1 (band 3)," *Journal of Biological Chemistry*, vol. 278, no. 52, pp. 52551-52558, 2003.

[14] S. Ficarra *et al.*, "Derangement of erythrocytic AE1 in beta-thalassemia by caspase 3: pathogenic mechanisms and implications in red blood cell senescence," *Journal of Membrane Biology*, vol. 228, pp. 43-49, 2009.

[15] A. A. Mahmoud *et al.*, "Assessment of subclinical renal glomerular and tubular dysfunction in children with beta-thalassemia major," *Children*, vol. 8, no. 2, p. 100, 2021.

[16] M. G. Shaalan, M. K. Hassan, H. J. Al-Shanoof, and L. M. Al Naama, "Renal dysfunction in pediatric patients in Iraq with β-thalassemia major and intermedia," *Cureus*, vol. 14, no. 9, 2022.

[17] M. Mohkam, B. S. Shamsian, A. Gharib, S. Nariman, and M. T. Arzanian, "Early markers of renal dysfunction in patients with beta-thalassemia major," *Pediatric nephrology*, vol. 23, pp. 971-976, 2008.

[18] A. Kassab-Chekir *et al.*, "Oxidant, antioxidant status and metabolic data in patients with beta-thalassemia," *Clinica Chimica Acta*, vol. 338, no. 1-2, pp. 79-86, 2003.

[19] V. Sen *et al.*, "Urinary early kidney injury molecules in children with beta-thalassemia major," *Renal failure*, vol. 37, no. 4, pp. 607-613, 2015.

[20] A. N. Saliba *et al.*, "Thalassemia in the emergency department: special considerations for a rare disease," *Annals of Hematology*, vol. 99, pp. 1967-1977, 2020.

[21] M. M. Islam *et al.*, "Distribution of β-thalassemia and other hemoglobinopathies in Bangladeshi university students and ready-made garment workers," *Risk Management and Healthcare Policy*, pp. 2707-2714, 2021.

[22] P. Pasqualetti, D. Colantonio, A. Collacciani, R. Casale, and G. Natali, "Circadian pattern of circulating plasma ACTH, cortisol, and aldosterone in patients with β-thalassemia," *European Journal of Endocrinology*, vol. 123, no. 2, pp. 174-178, 1990.

[23] P. Ott, "Membrane acetylcholinesterases: purification, molecular properties and interactions with amphiphilic environments," *Biochimica et Biophysica Acta (BBA)-Reviews on Biomembranes*, vol. 822, no. 3-4, pp. 375-392, 1985.

[24] L. Khodadadi, Q. Cheng, A. Radbruch, and F. Hiepe, "The maintenance of memory plasma cells," *Frontiers in immunology*, p. 721, 2019.

[25] K. Hofmann, A.-K. Clauder, and R. A. Manz, "Targeting B cells and plasma cells in autoimmune diseases," *Frontiers in Immunology*, vol. 9, p. 835, 2018.

[26] R. Leo *et al.*, "Multiparameter analyses of normal and malignant human plasma cells: CD38++, CD56+, CD54+, cIg+ is the common phenotype of myeloma cells," *Annals of hematology*, vol. 64, pp. 132-139, 1992.

[27] S. Z. Usmani *et al.*, "Clinical efficacy of daratumumab monotherapy in patients with heavily pretreated relapsed or refractory multiple myeloma," *Blood, The Journal of the American Society of Hematology*, vol. 128, no. 1, pp. 37-44, 2016.

[28] M. A. Dimopoulos *et al.*, "Daratumumab, lenalidomide, and dexamethasone for multiple myeloma," *New England Journal of Medicine*, vol. 375, no. 14, pp. 1319-1331, 2016.

[29] A. Palumbo *et al.*, "Daratumumab, bortezomib, and dexamethasone for multiple myeloma," *New England Journal of Medicine*, vol. 375, no. 8, pp. 754-766, 2016.

[30] M. Attal *et al.*, "Isatuximab plus pomalidomide and low-dose dexamethasone versus pomalidomide and low-dose dexamethasone in patients with relapsed and refractory multiple myeloma (ICARIA-MM): a randomized, multicentre, open-label, phase 3 study," *The Lancet*, vol. 394, no. 10214, pp. 2096-2107, 2019.

[31] M.-V. Mateos *et al.*, "Daratumumab plus bortezomib, melphalan, and prednisone for untreated myeloma," *New England Journal of Medicine*, vol. 378, no. 6, pp. 518-528, 2018.