


**Research Article**

# Cytogenetic Analysis associated with Clinical Hypothyroid Iraqi patients: an in vitro study

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Hypothyroidism is a common endocrine disorder characterized by a deficiency of thyroid hormones (THs), commonly as a result of insufficient hormone production or less frequently due to insufficient hormone action in target tissues. It is most commonly seen in women between the ages of 30 and 50. The current study aims to investigate the cytogenetic indicators in hypothyroidism patients of different ages and compare them with a control group of healthy women. The current study was conducted on 20 Iraqi female patients with hypothyroidism and compared them with a healthy control group to assess chromosomal aberrations. The mitotic index and chromosomal aberration index were higher in hypothyroid patients than in healthy controls, with a significant difference at a probability level of less than 5%.

**1. Introduction**

Hypothyroidism is a common endocrine disorder characterized by a deficiency of thyroid hormones (THs), commonly as a result of insufficient hormone production or less frequently due to insufficient hormone action in target tissues [1]. The prevalence of hypothyroidism is around 4.6% in the USA, with 4.3% and 0.3% attributed to subclinical and overt disease, respectively. Hypothyroidism is six times more frequent in women and White people [2]. The incidence increases with age as it is more common in people older than 60 years, and approximately 10% of women older than this age have subclinical hypothyroidism [2].

The most common risk factor of hypothyroidism is a personal or family history of autoimmune diseases such as celiac disease or type 1 diabetes [3]. Other risk factors linked with the disease include Down and Turner syndrome, relative selenium deficiency, insufficient or excessive iodine intake, and childhood overweight [4]. Globally, iodine deficiency is still the major etiology of the disease, while in areas where iodine intake is adequate, autoimmune thyroiditis is the predominant cause, followed by iatrogenic, drugs, pituitary, and hypothalamic diseases [5]. Clinical presentation of hypothyroidism can vary from mild to potentially life threatening signs or symptoms depending on factors such as onset of presentation, sex, age, pre-existing thyroid disease, etc. [6]. It presents most commonly with fatigue, weakness, cold intolerance, weight gain, constipation, periorbital edema, and dry and coarse skin [7].

Due to the lack of specificity of these manifestations, diagnosis is made by laboratory measurements of serum-free thyroxine (T4) and thyrotropin-stimulating hormone (TSH) levels [6]. Increased TSH levels indicate hypothyroidism, while free T4 can distinguish between overt and subclinical disease, presenting with low and normal levels, respectively [3].

Our body is made up primarily of somatic cells. It's possible that for our organism to maintain normal balance, these cells will need to proliferate. The cell cycle is a highly regulated process that drives growth. A cell eventually divides into two identical cells during this process, which takes one or two days. Cell death or unchecked cell proliferation that leads to cancer can be the outcome of cell cycle dysregulation [8]. Furthermore, cell development or death may be altered as a result of exposure to hazardous substances [9]. Since bone

marrow is the source of all blood cells, many studies that assess the immune system's activity and how various agents affect it rely on lymphocytes' capacity to proliferate in lymphoid organs and/or divide bone marrow cells [10]. The ratio of cells in a population going through mitosis to all cells is known as the mitotic index (MI) assay [11]. Consequently, this assay allows for detecting the effects of various physical and chemical agents on the mitotic response. Prior research has shown that chemicals, radiation, medications, and medicinal plants can all positively or negatively impact MI [11] and [12]. In the present study, we attempted to study the chromosomal changes of peripheral blood lymphocytes (PBLs) in patients with clinical and subclinical hypothyroidism, by measuring both mitotic index, blastogenic index and total chromosomal aberrations in patients and healthy groups.

## 2. Material and Method

### 2.1. Subjects

A 5 mL sample of venous blood was taken from 50 women suspected of having hypothyroidism, aged 32–47 years, after sterilizing the puncture site well with ethyl alcohol more than once to ensure obtaining a sterile sample. Ten women who were not infected and had no history of hypothyroidism were also taken. The sample was divided into two parts. The first part was placed in a sterile tube containing 10 microliters of anticoagulant heparin for cytogenetic tests, the second tube is free of any anticoagulant to conduct a thyroid hormone test. The blood is centrifuged at a speed of 5000 rpm for five minutes to obtain blood serum and conduct a T4, T3, and TSH test using a Japanese device (Abbott AxSYM) technique of enzyme immunoassay (EIA) is based on measuring the color intensity of the methyl umbelliferone complex resulting from the addition of umbelliferol methyl phosphate to a labeled phosphate alkaline conjugate. The absorbance value is proportional to the concentration of the hormone to be measured in the blood sample. The analysis result is obtained two minutes after placing the blood serum in the device and following the operating instructions provided by the supplier.

### 2.2. Chromosomal Analyses

#### Blood culturing

The blood culturing was carried out to obtain chromosomes according to the method mentioned in [13] by adding 0.5 mL of whole blood to 4.5 mL of complete culture medium (RPMI - 1640) prepared with 10 microliters of the stimulating substance phytohemagglutinin (PHA). The tubes were incubated in an inclined manner at a temperature of 37 °C for 70 hours. After the specified incubation period, the colchicine solution was added at a final concentration of 10 micrograms/mL and the tubes were returned to the incubator at a temperature of 37 °C for 2 hours to complete the culturing process for 72 hours. Then the culture was centrifuged at a speed of 3000 rpm for 10 minutes and after the filtrate was discarded, the precipitate was suspended in 5 mL of hypotonic calcium chloride solution, which was added gradually with continuous shaking. Then the tubes were returned to the incubator for another 45 minutes to get rid of the red blood cells by exploding them in the (KCL) solution and swelling the lymphocytes to be ready to prepare the chromosomes from them. Then the contents of the tubes were centrifuged at a speed of 3000 rpm for 10 minutes to get rid of the filtrate and suspend the precipitate with the cold fixative solution prepared immediately from mixing three parts of absolute methanol with one part of glacial acetic acid. This solution was added in drops to the wall of the tube with continuous shaking until a volume of approximately 5 mL was obtained. Then the centrifugation process was carried out at a speed of 3000 rpm. This process was repeated several times until the solution became colorless. Then the precipitated cells were suspended with 1–1.5 mL of the fixative solution, mixed using a clean, dry Pasteur pipette to become ready for dropped onto cold, moist slides.

A specific volume of the cells prepared above was taken, and at a height of about 80 cm, [7] spaced drops were dropped onto the glass slide to obtain good spreading of the chromosomes for easy observation of chromosomal changes.

#### Slide preparation and staining

The slides prepared in the previous section were air-dried. After they were completely dry, they were stained with Giemsa stain for two minutes, then washed with distilled water. During this time, both the blast transformation index (BI) and the mitotic index (MI) were calculated according to the equations shown below.

$$MI = \frac{\text{Mitotic cells}}{1000 \text{ cell}} 100\%$$

$$BI = \frac{\text{stimulated cells}}{1000 \text{ cell}} 100\%$$

Chromosomal aberrations = summation of total abnormalities within 25 mitotic cells.

## 3. Results And Discussion

Thyroid hormones for hypothyroidism and healthy group were determined Table 1, the results showed high serum level of TSH (30.05) than normal level (1.37). While we noticed a significant decrease in the (T3, T4) value in the patients group compared to the healthy group, this occurs according to the mechanism of negative feedback, due to the reduction of thyroid hormones (T3, T4) in the blood [14]. One of the most important reasons that may lead to hypothyroidism and low level of T3 and T4 hormones is the occurrence of immune reactions against the thyroid gland leading to the destruction of glandular cells. At first, the thyroiditis occurs and then damage the tissues usually ending up with the damage of the gland, as a result there is a deficiency in its ability to produce and release thyroid hormones [15]. The disease also happens as an effect of inadequacy or dysfunction of enzymes like the absence of Peroxidase or Deiodinase enzyme [15]. Modified expression of the deiodinases which is responsible for converting T4 into T3 in peripheral tissues and modified entry of thyroid hormone into tissue (damage membrane) may lead to hypothyroidism as well [16].

**Table 1:** Hormonal measurements in patients with hypothyroidism and group of healthy individuals

| Group                | Thyroid function test |             |               |
|----------------------|-----------------------|-------------|---------------|
|                      | T3 (ng/dl)            | T4 (µg/dl)  | TSH (µIU/L)   |
| Hypothyroid Patients | 37.35 ± 15.66         | 1.07 ± 0.42 | 30.05 ± 10.91 |
| Healthy              | 94.91 ± 12.29         | 8.18 ± 1.74 | 1.37 ± 0.38   |
| P-Value              | P < 0.05              |             | P < 0.05      |

**These results clearly show:**

- **TSH is significantly elevated** in patients compared to controls.
- **T3 and T4 are significantly reduced** in patients, far below the normal range.

Table 2 summarizes the numbers of mitotic cells per 1000 cells, blastogenic index per 1000 cells, and total chromosomal aberrations among 25 well-dividing cells in peripheral blood lymphocytes of Iraqi hypothyroidism patients compared with healthy controls. From the results can be observe cytotoxic and chromosomal aberrations in the patient's group through the significant decrease in the BI value (39.63±7.44) compared to the healthy group (61.35±8.21). The number and functional activity of peripheral blood lymphocytes and their ability to divide decreased (35.40) % in the patient's group, this means that (PBLs) lost more than a third of their ability to divide and differentiate, which is the second defense's line after macrophages. The mitotic index also suffering from significantly decreasing in the patient's group compared to the control group (22.36) % at (P<0.05), while the total chromosomal aberrations, The patient's group recorded a value (0.13±0.08) and we did not observe any chromosomal aberrations in the control group. The rate of blastogenic index and mitotic index in a tissue sample give a good bioindicator for their activity. This percent can be calculated by dividing the total number of cells in a high-power field (HPF) by the number of mitotic cells, patients that suffering from hypothyroidism recorded low functional activity for (PBLs), this decrease is not observed in Hashimoto's thyroiditis typically has an elevated mitotic index [17] This is because the inflammation and immune response associated with the disease lead to increased cell turnover. However, the mitotic index in Hashimoto's thyroiditis remains lower than in malignant thyroid tumors [18]. BI and MI may be a useful diagnostic marker associated with hypothyroidism. However, further research is needed to confirm this. The results of (TCAs) showed a significant increase (P<0.05) in the number of chromosomal aberrations represented by (chromosomal or chromated breaks, ring chromosomes, micronuclei) that could be formed compared to the control group, with 0.00 versus 0.13 respectively. Hormonal imbalance associated with hypothyroidism undoubtedly leads to a low both i number and effectiveness of human's lymphocytes. This imbalance may extend to the genetic material, as we saw in the current study, may be a cause of the development of any type of thyroid malignance. Hypothyroidism is considered a metabolic disorder because it affects the body's metabolism. It occurs due to a deficiency in the production of thyroid hormones, which regulate metabolism. This deficiency leads to a slowdown in many bodily functions, it raises levels of bad cholesterol (LDL), hyperglycemia due to increased insulin tolerance, decreased liver and kidney functions, accumulation of harmful toxic, and slowing down their excretion. These reasons may be a direct or indirect cause of the emergence and development of chromosomal aberrations, DNA damage, and DNA delayed repair mechanisms [16]. It is noteworthy that the level of increased chromosomal aberrations and decreased in BI and MI are positively related to the patient's age and duration of the disease. It is noted that patients who have been suffering from hypothyroidism for more than five years develop DNA damage and delayed repair mechanisms more than patients who have been suffering from hypothyroidism for less than five years Table 3 and 4, Figure 1. Hence, complete care must be taken with patients with hypothyroidism due to the possibility of developing malignant diseases as a result of permanent cellular stress, decreased metabolic activity [19] and [20].

**Table 2:** The frequency of BI, MI, and TCAs formation in Hypothyroidism patients and healthy control

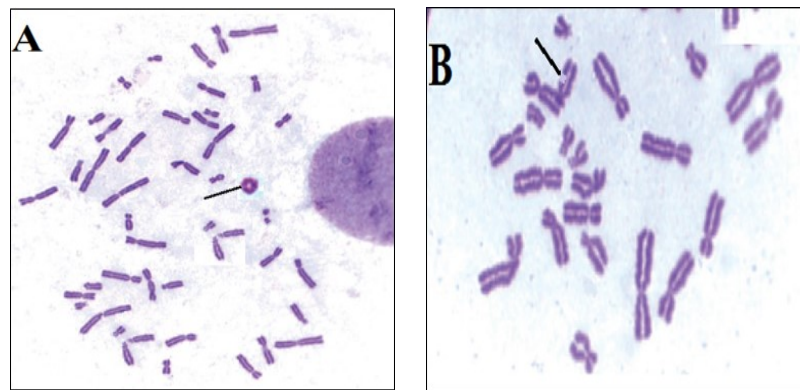
| Groups         | Blastogenic index (BI)% | Mitotic index (MI) % | TCAs      |
|----------------|-------------------------|----------------------|-----------|
| Hypothyroidism | 39.63±7.44              | 1.25±0.08            | 0.13±0.08 |
| Healthy        | 61.35±8.21              | 1.61±0.03            | 0.0       |
| P-Value        | 0.003                   |                      | P < 0.05  |

**Table 3:** The frequency of BI, MI, and TCAs formation in Hypothyroidism patients over 35 years of age

| Groups    | Blastogenic index (BI)% | Mitotic index (MI) % | TCAs      |
|-----------|-------------------------|----------------------|-----------|
| ≤35 Years | 41.22±3.16              | 1.41±0.02            | 0.15±0.01 |
| ≤45 Years | 33.38±5.11              | 1.11±0.01            | 0.17±0.02 |
| P-Value   | 0.007                   |                      | P < 0.05  |

**Table 4:** The frequency of BI, MI, and TCAs formation in Hypothyroidism patients suffered from the disease for more than five years

| Groups   | Blastogenic index (BI)% | Mitotic index (MI) % | TCAs      |
|----------|-------------------------|----------------------|-----------|
| >5 Years | 44.51±1.38              | 1.37±0.01            | 0.12±0.01 |
| <5 Years | 38.12±1.56              | 1.12±0.01            | 0.19±0.01 |
| P-Value  | 0.005                   |                      | P < 0.05  |



**Figure 1:** Chromosomal aberrations of peripheral blood lymphocytes (PBLs); (A) ring chromosome (B) Chromatid's break induced in hypothyroidism patients (100X)

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