Study the Effect of chemical treatments on the physiological and biochemical parameters of patients with cancerous diseases in Thi-Qar Governorate

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ABSTRACT

This research was designed to study the impact of chemotherapy on cancer patients' physiological and biochemical alterations in Thi-Qar Governorate. The study included 138 participants and 46 women with breast cancer divided into 23 samples taken (1-5 doses) and 23 took more than 5 doses. The treatment was Paclitaxel and Carboplatin, and 46 males with liver cancer divided into 23 samples taken (1-5 doses) and 23 took more than 5 doses. The treatment was Doxorubicin. 46 healthy individuals, 23 males and 23 females. The study was conducted in Al-Haboubi Hospital, Department of Oncology, in Thi-Qar Governorate, for the period between December 2022 - February 2023. With regard to blood parameters, the results showed a clear decrease in hemoglobin, red blood cells, white blood cells, and platelets in males and females who were treated with chemotherapy. As for liver enzymes, we notice an increase in ALT and AST in males with liver cancer more than in females with breast cancer. As for the ALP enzyme, we notice an increase in both sexes, and there is no significant difference between them. As for lipid, it is noted that there are clear changes, an increase in cholesterol and TG, a decrease in HDL in both sexes. For the hormones that are LH and E2, we notice a clear decrease in the value of the hormones compared to the normal level. For males, we notice a clear decrease in FSH and testosterone compared to the normal level.

Keywords: Cancerous disease, chemical treatments

INTRODUCTION

Cancer expresses a group of neoplastic diseases that are similar in their characteristics and similar in the pattern of their behavior, which arise from a single somatic cell, when this cell turns to become abnormal, grows and multiplies without control and deviates from the line of normal growth and reproduction, and instead of the death of these cells in their final phase, they continue to grow and multiply quickly and in an irregular manner, producing new abnormal cells that accumulate to form a cancerous tumor. This cell cancer transformation does not occur immediately or overnight (1). This transformation results from exposure to a number of factors that are classified as carcinogens (2). The danger of cancerous tumors, in addition to the abnormal structure and growth, lies in the ability of the cells of these tumors to invade and affect vital tissues or organs, whether nearby or distant, and may lead to destroy them (1), the chemotherapy is typically utilized for treating systemic illness.
By interfering with cell activity, which kills cancer cells or slows their growth, this anti-cancer drug is used to treat many cancers (3). Not all chemotherapeutic agents can cause the same side effect at same degree, it can rely on many effects, like type, length, dosage of the treatment and the health history of patient. The chemotherapeutic agents are usually given in a sessions, between every session, the patients should have a rest time to recover their strength (4). Fatigue and hair loss are two of chemotherapy’s most frequent side effects. simple bleeding and bruises Anemia (low red blood cell levels), infection, and nauseous and dizzy, the appetite shifts. digestive discomforta stomachache, throat, mouth, and tongue conditions that include sores and pain while swallowing Having peripheral neuropathy or other nerve problems may result in tingling, numbness, or pain, as well as changes to the skin, nails, and urine and bladder functions as well as renal issues. These changes can include dry skin and nail color. Changes in body composition, mood, libido, and sexual function, as well as issues with conception. (5).

**MATERIALS AND METHODS**

The preparation Of The Sample:

**Sample Collection**

For each of the pre-selected groups, five ml of blood were taken from the patient.

**Serum Preparation**

After the blood is drawn, we use 3 ml of the whole sample to begin the process of coagulation, which separates the serum from the blood, by placing it in a gel tube. To begin, we spun the gel tube at 4000 revolutions every minute (rpm) lasting five minutes in order to extract the serum.

**Whole Blood**

2 ml of blood are extracted from the individual’s body and placed in an EDTA tube, an anticoagulant tube, after being drawn.

Laboratory Experiments

**Complete Blood Count (CBC)**

This analysis is one of the important analyses to give a complete picture of the main components in the blood, which includes the number and concentration of red blood cells RBC, the number and types of white blood cells WBC, as well as the percentage of platelets PLT. In addition to the concentration of hemoglobin.

**Principle**

In this study used the device (GENEX count 60), Principle operation of the device is based on Electrical resistance for counting WBC, RBC and PLTs, figure(1-1)
Lipid Profile
Cholesterol Principle
Description of an enzymatic procedure by Allain et al(6)

Procedure
Manual method:
Reagent and samples should be let to sit at room temperature. Blending of (Reagent, Blank, Standard, Control, or specimen). At room temperature, let stand for 10 minutes; at 37 °C. For 5 minutes. Against a reagent blank, note absorbances between 480 and 520 nm at 500 nm. For 1 hour, color stays steady.

\[
\text{Cal.} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}
\]

Triglycerides Principle
Trinder reaction is connected to the Fossati and Principe method(7). The quantity of triglycerides in the material is directly proportional to the absorption measured at 500 (480-520 nm).

Procedure
Manual method
Reagent and samples should be left to sit at room temperature.

Blending. Allow to stand for 10 minutes at the temperature of the room or 5 minutes at 37° C. Compare the absorbance at 500 nm (480-520) to a blank for the reagent. For one hour, color remains steady.

\[
\text{Cal.} = \frac{\text{Abs (Assay)}}{\text{Abs (Calibrator)}} \times \text{Calibrator concentration}
\]

Low Density Lipoprotein LDL Principle
It is possible to calculate the level of LDL through an equation

\[
\text{LDL} = \text{cholesterol} - \text{HDL} - \text{VLDL}
\]

Very Low Density Lipoprotein VLDL Principle
It is possible to calculate the level of VLDL through an equation developed for this purpose.

Procedure
VLDL = Triglycerides / 5

Liver Enzymes Aspartate Aminotransferase AST - GOT Principle
Reitman and Frankel modified the colorimetric technique used by Tonhazy, White, and Umbreit to determine the activity in serum. (8).

Procedure
R2 Reagent (1 mL) At 37 °C, incubate for 5 minutes. Add: 200 mL of serum then combine and incubate for exactly one hour at 37 °C. Once blended, let Reagent R3 (1 mL) remain at room temperature for 20 minutes. NaOH, 0.4 N (10 mL), add.
After 5 minutes, measure the amount of absorption at 505 nm in comparison to water.

Cal.

Calculate the result as enclosed Standard Curves (batch specific)

**Alanine Aminotransferase ALT - GPT Principle.**

Reitman and Frankel modified the colorimetric technique used by Tonhazy, White, and Umbreit to determine the activity in serum. (8).

**Procedure**

R2 Reagent (1 mL) At 37 °C, incubate for 5 minutes. Add 200 mL of serum then combine and incubate for exactly one hour at 37 °C. Once blended, let Reagent R3 (one milliliter) rest at room temperature for 20 minutes. NaOH 0.4 N (10 mL) is added.

After 5 minutes, measure the amount of absorption at 505 nm in comparison to water.

Cal.

Calculate the result as enclosed Standard Curves (batch specific).

**Alkaline Phosphatase (ALP) Principle**

ALP activity is determined colorimetrically. (9).

Cal.

Results (Kind and King unit):

Size of the enzyme that, under the circumstances of the reaction, releases 1 mg of phenol in 15 minutes at 37°C.

Or Result (IU/L) = 7.09 x Result (Kind and King Unit/100 mL)

**Component of Estradiol Hormone Kit Test Procedure**

Before beginning the test, bring all of the substances, serum standard calibrators, and controllers to ambient temperature (20-27°C). Prepare duplicate microplate wells for every serum reference calibrator, control, and patient sample. Pipette the appropriate serum reference calibrator, control, or sample into the corresponding well, totaling 0.025 mL (25 L). Each well should contain 0.050 mL (50 l) of estradiol biotin reagent. For 20 to 30 seconds, gently spin the microplate to blend. 30 minutes should be spent covered and at room temperature.

To each well, add 0.050 mL of the estradiol enzyme reagent. Directly on top, add the reagents that have been dispersed throughout the wells. For 20 to 30 seconds, gently spin the microplate to blend. 90 minutes should pass at room temperature under cover. Decantation or aspiration are two methods for getting rid of the microplate's contents. When decanting, wipe the plate dry with absorbent paper. Buffer (see Section on Reagent Preparation). For a total of two (2) additional washes, repeat this procedure. Add 0.100 mL (100 l) of substrate solution to each well. Reduce variation in reaction times between wells by adding chemicals in the same order every time. After adding the substrate, do not shake the plate. Incubating should take place at room temperature for 20 minutes. Each well should receive 0.050ml (50l) of the stop solution, which should be mixed there gently for 15 to 20 seconds.

Using a reference wavelength between 620 and 630 nm, calculate the absorbance in each well at 450 nm. The findings should be read fifteen (15) minutes after the stop solution was administered.

**Calculation Of Results**

The content of estradiol in unknown specimens is determined using a dosage response curve.

**Component Of Luteinizing Hormone Kit Test Procedure**

Prepare duplicate microplate wells for each serum standard calibrator, oversight, and patient sample being evaluated. Pipette 0.050 ml (50 l) of the selected serum standard calibrator, oversight, or sample into the designated well. Each well should contain 0.100 mL (100 l) of LH-Enzyme Reagent. To mix and cover, lightly spin the microplate for 20 to 30 seconds. The
incubation period must be 60 minutes at room temperature. The contents of the microplate must be taken out via aspiration or decantation. If decanting, use absorbent paper to dry the plate. To decant (tap and blot) or aspirate 0.350ml (350l) of wash buffer, see the section on preparing reagents. Repeat the procedure two more times for a total of two (2) washes. A total amount of 0.100 ml (100 l) of working substrate solution has to be supplied to each well (see the section on reagent preparation). Always To minimize variations in reaction times between wells, use the chemicals in the same sequence. Incubate for a total of fifteen minutes at room temperature. Each well should receive 0.050ml (50 l) of the stop solution, which should be mixed there gently for 15 to 20 seconds. Utilizing a microplate reader, determine the amount of absorption in each well at 450 nm (reducing well defects by using a standard wavelength of 620–630 nm). After thirty (30) minutes of applying the stop solution, the findings should be read.

**Cal. Of Results**

The level of luteinizing hormone in unknown specimens is determined using a dosage response curve.

**Male Hormones**

**Component Of Testosterone Hormone Kit:**

**Test Procedure**

Before beginning the test, bring all of the substances, serum calibrators, and standards to the ambient temperature (20–27°C). Each serum reference, control, and patient sample that will be tested in triplicate should have its microplate wells prepared. Pipette 0.010 mL (10L) of the pertinent serum guidance, control, or sample into the relevant well. The Testosterone Enzyme Reagent, that is prepared to use, should be added to each well at a rate of 0.050 mL (50 l). For 20 to 30 seconds, gently whisk the microplate to blend. Add Testosterone Biotin Reagent in the amount of 0.050 mL (50 l) to each well.. Gently stir the microplate for 20 to 30 seconds to combine. For 60 minutes, cover and leave at room temperature. Removing the microplate's contents via aspiration or decantation is necessary. If decanting, dry the plate with absorbent paper. 0.350ml (350l) of wash buffer should be decanted (tap and blotted) or aspirated (see the section on preparing reagents). For a total of two (2) additional washes, repeat this procedure. Add 0.100 ml (100 l) of the working substrate solution to each well.

After Adding The Substrate, Do Not Shake The Plate. Incubate for fifteen (15) minutes at room temperature. Each well should receive 0.050ml (50l) of the stop solution, which should be mixed there gently for 15 to 20 seconds. At 450 nm, calculate the absorbance in each well of a microplate reader. Use a reference wavelength of 620–630 nm to reduce well flaws. Within thirty (30) minutes of applying the stop solution, the findings should be read.

**Cal. Of Results**

A dose response curve is used to estimate the concentration of the hormone testosterone in unidentified specimens.

**Component Of Follicle Stimulating Hormone Kit**

**Test Procedure**

Before beginning the test, bring all of the reagents, serum calibrators, and standards to room temperature (20-27°C). Each serum guidance, control, and patient samples that will be tested in triplicate should have its microplate wells prepared. In the appropriate well, pipette 0.010 mL (10L) of the relevant serum reference, control, or sample. Add 0.050 mL (50 l) of the FSH-Enzyme Reagent, which is ready for use, to each well. Gently stir the microplate for 20 to 30 seconds to combine. Add 0.050 mL (50 l) of Testosterone Biotin Reagent to each well. Gently stir the microplate for 20 to 30 seconds to combine. For 60 minutes, cover and leave at room temperature. Removing the microplate's contents via aspiration or decantation is necessary. If decanting, dry the plate with absorbent paper. 0.350ml (350l) of wash buffer should be decanted (tap and blotted) or aspirated (see the section on preparing reagents). For a total of two (2) additional washes, repeat this procedure. Add 0.100 ml (100 l) of the working substrate solution to each well.
After Adding The Substrate, Do Not Shake The Plate. Incubate the sample for around fifteen (15) minutes at room temperature. Each well should receive 0.050ml (50l) of the stop solution, which should be mixed there gently for 15 to 20 seconds. At 450 nm, calculate the absorbance in each well of a microplate reader. Use a reference wavelength of 620–630 nm to reduce well flaws. Within thirty (30) minutes of applying the stop solution, the findings should be read.

**Cal. Of Results**
The levels of the follicle-stimulating hormone in unidentified specimens are determined using a dosage response curve.

**RESULTS AND DISCUSSION**
Results of Hematological Parameters in Breast Cancer, Liver Cancer and Control Group

| TABLE 1: Estimation hematological parameters in cancer patients and control group |
|---------------------------------|--------------|--------------|--------------|--------------|
| Hematological Groups            | WBC*10^3     | LYM%         | RBC*10^6     | Hb gm/dl     |
| Mean ± SD                       |              |              |              |              |
| Breast Cancer                   | 3.26 ± 0.42^a| 18.2 ± 0.68^a| 3.05 ± 0.28^a| 9.03 ± 0.79^a|
| Liver Cancer                    | 3.14 ± 0.45^b| 17.2 ± 1.13^b| 2.75 ± 0.30^b| 8.78 ± 0.83^b|
| Control                         | 7.86 ± 1.71^c| 29.7 ± 5.60^c| 4.71 ± 0.54^c| 13.4 ± 1.36^c|
| p. value                        | < 0.01**     | < 0.01**     | < 0.01**     | < 0.01**     |
| LSD                             | 0.43         | 1.36         | 0.16         | 0.42         |

The table's results also revealed a significant difference between the red blood cells of cancer patients—male or female—and healthy subjects, and that the cause of this difference is chemotherapy, which affects the activity of the bone marrow that produces these cells. This is in line with the Salem and his team's work from 2006(12)(13), which shows a reduction in erythrocytes. When compared to healthy patients, it was anticipated that both men and females with cancer would have a substantial difference (Hb). This decline has been attributed to cancer-related anemia, which worsens with chemotherapy dosages, according to studies (14) (15)(16).

This finding suggests that PLT may be much lower in cancer patients' male and female compared to their healthy counterparts. Studies (14) (15)(16) have shown that chemotherapy-related variations may be considerable.

As for the results of Liver Function Parameters in Breast Cancer, Liver Cancer and Control Group:
TABLE 2: Estimation liver function parameters in cancer patients and control group

<table>
<thead>
<tr>
<th>LFT Groups</th>
<th>ALT IU/L Mean ± SD</th>
<th>AST IU/L Mean ± SD</th>
<th>ALP IU/L Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast Cancer</td>
<td>40.0 ± 3.29</td>
<td>38.3 ± 3.75</td>
<td>146.5 ± 2.91</td>
</tr>
<tr>
<td>Liver Cancer</td>
<td>46.5 ± 3.07</td>
<td>99.1 ± 3.45</td>
<td>147.2 ± 2.13</td>
</tr>
<tr>
<td>Control</td>
<td>20.1 ± 6.49</td>
<td>33.9 ± 8.11</td>
<td>76.01 ± 19.0</td>
</tr>
<tr>
<td>p. value</td>
<td>&lt; 0.01**</td>
<td>&lt; 0.01**</td>
<td>&lt; 0.01**</td>
</tr>
<tr>
<td>LSD</td>
<td>1.88</td>
<td>4.42</td>
<td>4.62</td>
</tr>
</tbody>
</table>

According to the findings of the present study, one of the most frequent signs in the clinical practice of liver cancer patients is an increase in the levels of liver enzymes (17). The enzymes ALT, AST, and ALP were found to be significantly elevated when we evaluated the activity of enzymes in liver cells. When compared to the enzymes produced by healthy liver cells, ALT, AST, and ALP are released in significant amounts into the blood by damaged liver cells. (18) hypothesize that the reason why AST, ALT, and ALP levels react quickly and increase quickly when hepatocytes are harmed is due to the immediate release of reservoirs of aminotransferases into tumor cells and their short half-life of days.

Table (1-2) is also shown increase in liver enzymes in breast cancer patients compared to healthy people and the reason for this is the side effects of treatment that cause hepatotoxicity and it is a common occurrence. (19) It is consistent with a study (20) in which he reported elevated liver enzymes for women with breast cancer.

Hepatotoxicity from chemotherapy occurs frequently from an unpredictable or idiosyncratic reaction (21).

The use of drugs that are purposefully designed to be cytotoxic has unavoidable negative effects in the complicated realm of cancer treatment (22). Since many of these medications are metabolized primarily in the liver, the liver-drug interaction must be taken into consideration when prescribing chemotherapy (23). The central and sublobular veins of the liver may get blocked by a variety of antineoplastic medications, leading to SOS. Common symptoms include jaundice, hepatomegaly, abdominal discomfort, and ascites (24).

As for the results of Lipid Profile Parameters in Breast Cancer, Liver Cancer and Control Group:

TABLE 3: Estimation lipid profile parameters in cancer patients and control group

<table>
<thead>
<tr>
<th>Lipid profile Groups</th>
<th>CHOL mg/dl Mean ± SD</th>
<th>TG mg/dl Mean ± SD</th>
<th>HDL mg/dl Mean ± SD</th>
<th>LDL mg/dl Mean ± SD</th>
<th>VLDL mg/dl Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast Cancer</td>
<td>214.0 ± 3.89</td>
<td>188.4 ± 3.93</td>
<td>29.5 ± 1.56</td>
<td>146.6 ± 2.71</td>
<td>37.7 ± 0.78</td>
</tr>
<tr>
<td>Liver Cancer</td>
<td>217.3 ± 5.13</td>
<td>191.7 ± 4.07</td>
<td>29.8 ± 1.52</td>
<td>149.1 ± 3.24</td>
<td>38.3 ± 0.81</td>
</tr>
<tr>
<td>Control</td>
<td>156.7 ± 18.1</td>
<td>115.0 ± 21.1</td>
<td>43.5 ± 6.24</td>
<td>90.2 ± 18.2</td>
<td>23.0 ± 4.23</td>
</tr>
<tr>
<td>p. value</td>
<td>&lt; 0.01**</td>
<td>&lt; 0.01**</td>
<td>&lt; 0.01**</td>
<td>&lt; 0.01**</td>
<td>&lt; 0.01**</td>
</tr>
<tr>
<td>LSD</td>
<td>4.58</td>
<td>5.21</td>
<td>1.57</td>
<td>4.40</td>
<td>1.04</td>
</tr>
</tbody>
</table>

A lipid profile is a collection of blood tests used to measure the blood’s triglyceride and cholesterol levels. These are nothing more than fatty acids in your blood. Your arteries may get blocked by unhealthy cholesterol levels, which raises your risk of heart disease and stroke (25).

We notice in Table (1-3) a clear increase in fat compared to the control group, and the reason is due to the effect of chemotherapy on the level of fat regarding breast cancer With the exception of the decline in HDL-C, the cholesterol rose...
considerably throughout chemotherapy. This rise in cholesterol in chemotherapeutic patients may be partially explained by the rise in thyroxin levels during chemotherapy that our earlier investigations had discovered (26). Lipid abnormalities in cancer patients may be an acute-phase reaction brought on by the secretion of cytokines by inflammatory cells around the tumor or by the tumor cell itself (27).

Although adjuvant chemotherapy increases breast cancer patients’ overall and disease-free survival, mounting data points to the possibility that chemotherapy may also have a major impact on their metabolic condition (28). In this research, we found that breast cancer patients receiving adjuvant chemotherapy had some substantial metabolic alterations, including an increase in total cholesterol, triglycerides, LDL cholesterol, and a reduction in HDL cholesterol.

The high levels of these lipids (cholesterol, TG, LDL, and low HDL) were validated in investigations by Sharma et al. (2016) and Wei Tian et al. (2019), which are compatible with this finding.

There are still gaps in our understanding of how certain lipids change the metabolic environment in liver illnesses, which is relevant to liver cancer.

Chemotherapy impacts lipids by a variety of yet-unknown methods, but one of these processes was attained by (29) doxorubicin and was linked to a reduction in ABCA1 mRNA transcription. In their cell model, doxorubicin-induced general impairment led to a dose-dependent 20–30% cholesterol efflux. Doxorubicin-treated cells in a research using a mouse model showed elevated cholesterol levels.

As for the results of Hormones Parameters in Breast Cancer and Control Group

**TABLE 4:** Estimation hormones in breast cancer women and control group

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Breast Cancer No. 46 Mean ± SD</th>
<th>Female Control No. 23</th>
<th>p. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>0.86 ± 0.08</td>
<td>3.78 ± 1.50</td>
<td>&lt; 0.01**</td>
</tr>
<tr>
<td>E₂</td>
<td>21.7 ± 4.16</td>
<td>180.6 ± 58.7</td>
<td>&lt; 0.01**</td>
</tr>
</tbody>
</table>

The current results in Table 1-4 indicated a significant decrease in the concentration of luteinizing hormone and estrogen hormone in women with breast cancer compared to breast cancer patients and female control group. The reason for this decrease is for several reasons. First, in terms of age, females with cancer whose age is more than 45, there is actually a decrease in the concentration of hormones compared to healthy subjects whose age is less than 40 so the percentage of hormones is normal, and this is consistent with a study (30). And also the reason for this decline, and it is the main reason, is the side effect of chemotherapy also on the reproductive organs, including the ovaries, along with the levels of hormones in your body, and an imbalance may occur in the levels of estrogen and LH in your body because of the way chemotherapy affects your endocrine system. This was confirmed by (31). The results of the current study do not agree with the results of (32)(33). pointed out the importance of the role of estrogen in stimulating and growing cancer cells and raising its serum level in breast cancer patients. When normal cells are transformed into cancerous cells, they may often retain in their nucleus what we call the estrogen and progesterone ne receptor protein within the nucleus. Therefore, same hormones that support healthy breast cell growth and survival may also support the growth and proliferation of cancerous cells.
Study the Effect of chemical treatments on the physiological and biochemical parameters of patients with cancerous diseases in Thi-Qar Governorate

**TABLE 5:** Estimation hormones in Liver cancer women and control group

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Liver Cancer No. 46</th>
<th>Male Control No. 23</th>
<th>p. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>0.83 ± 0.08</td>
<td>9.56 ± 2.90</td>
<td>&lt; 0.01**</td>
</tr>
<tr>
<td>Testosterone</td>
<td>1.76 ± 0.23</td>
<td>5.55 ± 1.82</td>
<td>&lt; 0.01**</td>
</tr>
</tbody>
</table>

The current results indicated in Table (1-5) to a significant decrease in follicle stimulation hormone and testosterone hormone in males with liver cancer compared to healthy subjects, and that the first reason lies in the age difference the patients liver cancer were aged (49-72 years) compared to healthy subjects who were Their ages are younger (20-42) and that testosterone decreases with age and BMI and impaired QoL (34).

Chemotherapy may cause problems and disorders in some glands so that it may contribute to a deficiency in the production of some glands for hormones and damage to the tissues of some glands (35).

**CONCLUSION**
The current results of this research indicate that chemotherapy, despite the benefit of this treatment in reducing and eliminating cancerous tumors, has significant side effects, which is that it affects blood parameters (red blood cells, white blood cells and platelets) in addition to its effect on liver functions and enzymes. (AST, ALT, ALP) in addition to its effect on the lipid profile (cholesterol, triglycerides, HDL, LDL, and VLDL) as well as hormones. The reason for this is that chemotherapy is a systemic treatment that attacks cancer cells and normal cells as well.

**ACKNOWLEDGEMENTS**
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**REFERENCES**
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