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Estimation of Calcitonin Hormone Levels and its Relation with Tyrosine Kinase Enzyme in Thyroid Cancer Iraqi Patients

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Abstract

Medullary thyroid cancer accounts for 5% of all cases of thyroid cancer. It develops from the C-cells of the thyroid. Thus, in addition to its destructive impact on our health, cancer also significantly increases the overall healthcare burden. C-cells manufacture the hormone calcitonin, which plays a somewhat modest part in the process of bone development as well as blood calcium levels. That is because it holds the role of a poor substitute excreted if the thyroid is removed, the effect of calcitonin is weaker than that of thyroid hormone. Luckily, serum calcitonin concentrations that are higher than normal can help make a diagnosis and find out about a thyroid cancer recurrence. In contrast to almost all other thyroid type cancers, medullary thyroid cancer does not assimilate RAI and so the best way of saving the patient's life is to have the surgery done just in time when the tumour has not yet spread over the body. The greatest risk factor for medullary thyroid cancer is the occurrence of medullary thyroid cancer or MEN syndrome (multiple endocrine neoplasia syndromes) in families, up to 20% of cases carry a genetic flaw. Among other things, the patient screening for the eponymous mutation of the RET proto-oncogene should be given first priority. There are two forms of MEN syndrome. MEN 2A when the tumours develop in the medulla of the thyroid, adrenal and parathyroid glands. MEN 2B when it contains medullary thyroid carcinoma, pheochromocytoma, and multiple ganglioneuromas of the autonomic nervous system.

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1. Introduction

Medullary Thyroid Carcinoma is a proto-type of cancer which grows from parafollicular cells [C cells] of the thyroid gland. Medullary thyroid cancer, being a PEA (parathyroid Enteric Anterior Medullary) tumor, produces calcitonin; in addition, the most specific feature of this tumor is an elevated level of calcitonin in the patient's serum. It is made up of the C cells that are descended from cells deriving from the medullary origin of neural crest. The molecular pathogenesis of thyroid cancer is now fully understood. This has also led to genetic profiling that presents personalized risk stratification of the patients. Mutations that are at the higher risk of the development of the disease are molecular targets of therapy [1]. Prophylactic thyroidectomy is recommended for patients with mutations that put them at high risk. The FDA has approved several TKIs in advanced and growing metastatic medullary thyroid cancer cases [2]. Medullary Carcinoma is a thyroid cancer which account between 4% and 10% of all the thyroid cancers in the world. Some individuals with sporadic medullary thyroid cancer reach the peak incidence age of fifth or sixth decade unlike those developing such cancer type as a result of MEN 2A or MEN 2B which affect patients almost a decade earlier. Medullary thyroid cancer diagnosed by the existence of oval or round cell nests with fibrovascular stroma. Unlike other types of lumps that develop from the follicles, the cancerous mass develops from the parafollicular C cells of the thyroid. In medullary thyroid cancer, presented as a thyroid nodule in the upper portion (the region with predominant C cell localization) accounts for approximately 3/4 to 2/3 of the patients. In up to

70% of all involved patients will have enlarged lymph nodes in the neck region, and in a few patients the compression of adjacent structures (e.g. muscles that move pharynx or vocal cords) may result in dysphagia, hoarseness, or breathing difficulty. Even within the first 15%, patients can have metastases developed already when they are usually only diagnosed. This type of cancer usually spreads from thyroid origin to metastases in the liver, bone, lung, and brain. Calcitonin is the hormone produced by the thyroid gland when the calcium levels are too high in the body. About ten percent of patients on this drug experience diarrhea [3, 4]. The study links tyrosine kinase to the role of the tyrosine kinase inhibitor in this population of cases which are impossible to surgically remove and symptomatic. This class of drugs, the tyrosine-kinase inhibitors (TKI) such as vandetanib and cabozantinib may be used in this type of cases. Vandetanib (an oral, independent receptor kinase inhibitor) inhibits RET, EGFR, and VEGFR. In a phase III study that consisted of 331 subjects with advanced, inoperable, or metastatic medullary thyroid cancer, patients experienced prolonged PFS ($p < 0.01$) compared to placebo [5]. Capozantinib, an oral multi-kinase inhibitor, also has neoplasm progression control by restraining MET, RET, and VEGFR2 The outcome of this EXAM Phase III study has been significant in that there is an observed improvement in PFS in medullary thyroid cancer with an advanced or metastasised stage. Tyrosine kinase is the enzyme that catalysis the transfer of phosphate into the tyrosine residues in different macromolecules. The receptor tyrosine kinases generate an activation signal when its extracellular domain forms a bond with a specific ligand. These (e.g. EGF, PDGF etc.) are extracellular signalling molecules that bind to receptors and cause receptor dimerization (except the Insulin receptor). Each ligand employs a unique type of mechanisms that it utilizes in order to achieve the stable duplex configuration. One ligand may bind with two receptor molecules to form 1: The formation of two ligand receptor complexes. For example 2 growth hormone and 2 growth hormone receptor with the result that ligand dimerization is realized rather easily i.e. VEGF and VEGFR. This is ensured by receptor–receptor interactions that also promote the dimer stabilization. Another way this pathway is activated is through the interaction of proinflammatory cytokines. Certain ligand-receptor interactions do not only require the presence of ligands but also requires the presence of these molecules such as heparin sulfate proteoglycans (HSPG) to afford stability. Moreover, FGFs cannot activate FGFR complex without heparin sulfate proteoglycans (HPSG)[6].This study focuses on studying the levels of the calcitonin hormone and its relationship with the levels of tyrosine kinase enzyme and their effect on thyroid cancer patients.

2. Experimental Procedures

The research topic was conducted in accordance with the principles described in the World Medical Association Declaration of Helsinki, after following approval from the ethics committee in health ministry (ethical number 4287/3/2 at 19/11/2023). The study was undertaken independently, without any financial support.

2.1. Patients' Inclusion Criteria

In our study, we included 70 patients over the age of 18 who were diagnosed with thyroid cancer on the basis of the 2003 Rotterdam diagnostic standards. Furthermore, we divided them into two groups, one with chemical treatment and another without treatment, we included 20 healthy volunteers matched with gender and age and without any tumor disease who served as a control group. The Rotterdam diagnostic criteria for TC include the presence of ultrasonographic evidence, and biochemical test indicates high levels of calcitonin hormone and tyrosine kinase enzyme. Individuals who met of one out of the two criteria were classified as having thyroid cancer (TC).

2.2. Calcitonin hormone

The samples, kit reagents, and microplate that were set aside for the investigations were given time to reach the ambient temperature. The hormone Assay (ELISA) method with the assistance of a kit from Bioassay Technology Laboratory (Catalog No: EA0142Hu, China) was used to measure the concentration of human calcitonin hormone in the sample. This kit utilizes the biotinylated double sandwich approach for measurement. This kit utilizes a microplate that has been pre-coated with pure rat monoclonal calcitonin antibody. 50 microliters of calcitonin standards (48, 24, 12, 6, and 3 nanograms per milliliter) and 40 microliters of samples were introduced into the wells. The samples were supplemented with 10 µL of biotinylated anti- calcitonin antibody. Next, 50 µL of strepdavin-HRP was introduced to both the samples and standards, and the mixture was placed in a Sanyo Sterilizer incubator from Japan, Keeping the temperature at 37 °C for a period of 1 hour. After the incubation period ended, ELISA (Biotek ELx50, USA) underwent a cleaning process using a dedicated washing apparatus. Subsequently, 50

microliters of solutions containing Chromogen B and Chromogen A were introduced and kept a temperature at 37^{0}C degrees Celsius for a period of time of 15 minutes under light-restricted conditions. The reaction was halted by subjecting it to darkness to facilitate color production, and subsequently introducing an acidic solution. The color intensity was measured at a wavelength of 450 nm using an ELISA reader (Biotek ELx800, USA). Calcitonin levels were determined using typical graphical methods, figure 1 [7].

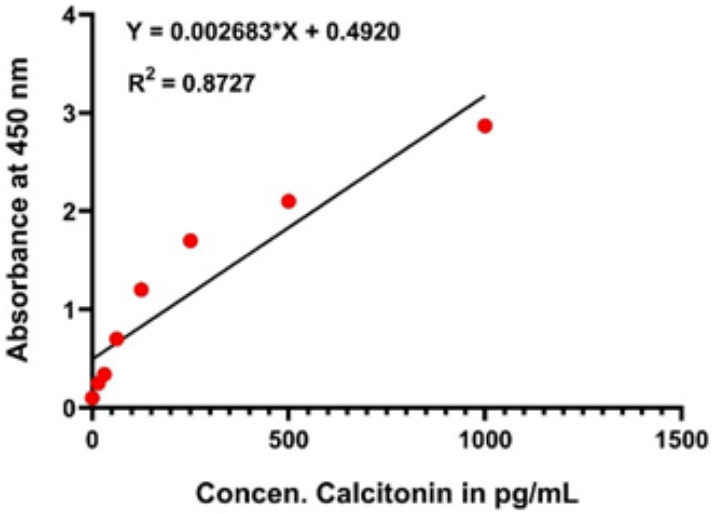

Figure 1. Human Calcitonin ELISA Standard Curve.

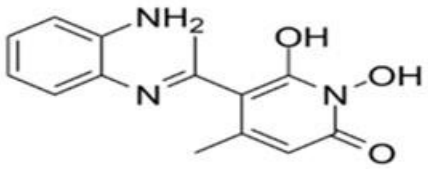

Figure 2. Calcitonin hormone chemical structure.

2.3. Tyrosine kinase

The samples, kit reagents, and microplate that were set aside for the investigation were let to reach the ambient temperature. by using an enzyme-linked immunosorbent assay (ELISA) method with the use of a kit provided by Bioassay Technology Laboratory (Catalogue No: E6022Hu, China) The level of human tyrosine in samples were measured .This kit utilizes the biotinylated double sandwich approach for measurement. This kit uses a microplate that has been pre-coated with pure rat monoclonal Ty antibody. 50 microliters of tyrosine standards (2400; 1200; 600; 300 and 150 nanograms per liter) and 40 µL of samples were added to the wells. Samples were supplemented with 10 µL of biotinylated anti-Ty antibody. Next, 50 µL of strepdavin-HRP was introduced to both the samples and standards. The mixture was then placed in a Sanyo Sterilizer incubator from Japan and Kept at a constant temperature of 37 °C for a period of 1 hour. Once the incubation period concluded, ELISA (Biotek ELx50, USA) underwent a cleaning process using a dedicated washing apparatus. Subsequently, 50 µL of Chromogen A and Chromogen B solutions were introduced and placed in an incubator set at a temperature of 37 degrees Celsius for duration of 15 minutes in a light-restricted environment. The reaction was halted by subjecting it to darkness to facilitate color production, and subsequently introducing an acidic solution. The color intensity was quantified using a spectrophotometer at a specific wavelength of 450 nm using an ELISA reader (Biotek ELx800, USA). Ty levels were determined using standard graphical methods, as shown in figures 3 [8].

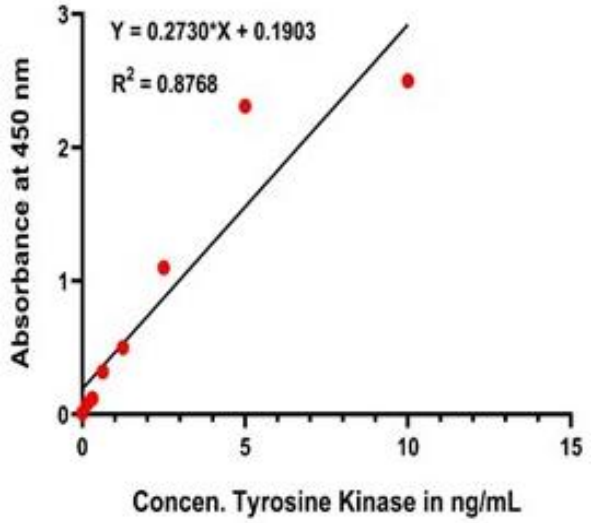

Figure 3. Human tyrosine kinase ELISA Standard Curve.

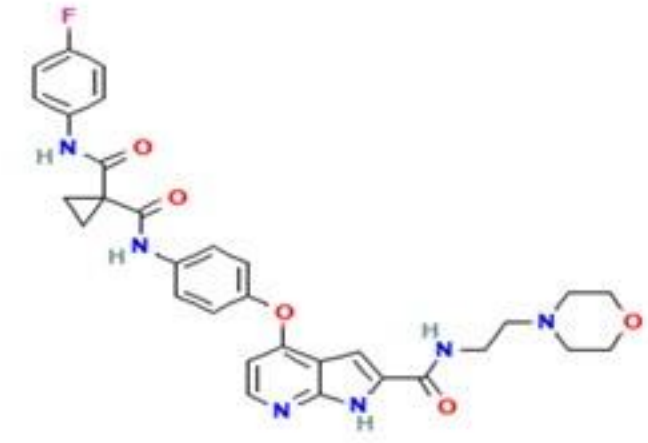

Figure 4. Tyrosine kinase chemical structure.

2.4. Statistical Analysis

Data were analyzed using Graph Pad Prism software version 8.0.2 (San Diego, California, USA). Means from three or more groups were compared using one-way analysis of variance (ANOVA). A post hoc test (Tukey's test; performed after ANOVA) was used to determine the statistical significance of difference between two groups. Results were presented as mean ± standard deviation (SD)...A *p*-value <0.05 was considered statistically significant. The receiver operator characteristics (ROC) curve analysis was used to assess the abilities of the markers in discriminating between treated thyroid cancer patients and untreated thyroid cancer patients and measuring specificity and sensitivity of the studied marker.

3. Result and Discussion

a. Calcitonin

Table 1 presents the mean ± SD of serum Calcitonin levels across different groups. In the control group, the average concentration of Calcitonin is 924.9 pg/mL, while untreated thyroid cancer (TC) patients show a significantly higher concentration of 1733.3 pg/mL. Treated TC patients exhibit an intermediate level of 1194.6 pg/mL. The reported p-value, < 0.0001, indicates a statistically significant difference in Calcitonin levels among the groups.

Table 1. The mean ± SD for calcitonin.

Parameters(pg/ml)	Control N=(25)	TC Untreated (n=35)	TC Treated (n=35)	P Value
Calcitonin	924±343.2	1733.3±518.3	1194.6±426.4	<0.0001

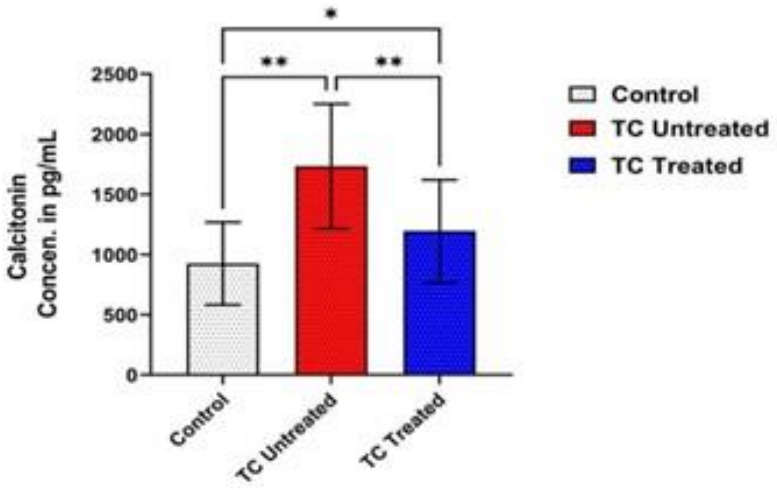

Figure 5. Calcitonin hormone concentration in study groups.
*single star indicates the presence of significant differences between the study parameters,
** double stars indicate the presence of high significant differences between the study parameters.at P value<0.0001

Figures 5 examining the hormone calcitonin levels unveils distinct patterns among different cases, where there was a significant difference between TC Treated and TC Untreated, and highly similar results between TC Treated and control. In the control group, the average concentration of calcitonin was 900 pg/mL, whereas untreated thyroid cancer patients exhibited a substantial elevation to 1700 pg/mL. Treated thyroid cancer patients displayed an intermediate concentration of 1100 pg/mL. These findings suggest a potential association between calcitonin levels and thyroid cancer, with untreated patients demonstrating the highest concentrations. These results lead to the understanding that says the calcitonin hormone levels can be a sign for TC disease. As mentioned in the study of [9] and [10].

Promising diagnostic potential. The analysis yielded a sensitivity of 98.31% and specificity of 96% at a cutoff value of >1212.5 pg/mL. The Area Under the Curve (AUC) was calculated to be 0.9966, indicating strong discriminatory power between thyroid cancer cases and controls. Additionally, the associated p-value, which was <0.0001, underscores the statistical significance of these findings (figure 6)

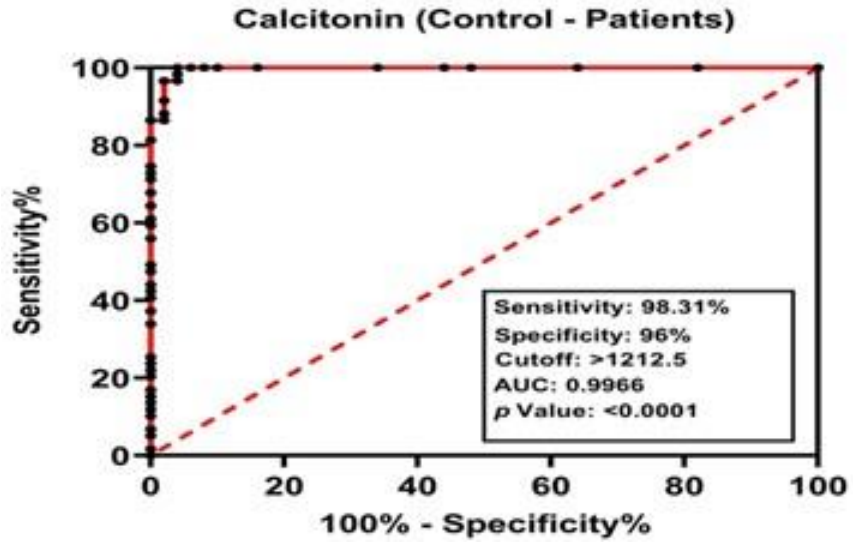

Figure 6. ROC Plot showing the summary curve of basal reported calcitonin cut-off values.

b. tyrosine kinase

Table 2. The mean ±SD for Tyrosine kinase.

Parametes(pg/ml)	Control (n=25)	TC Untreated (n=35)	TC Treated (n=35)	P Value
Tyrosine kinase	3.37±1.0	17.79±5.54	7.63±1.8	<0.0001

Table 2 presents the mean ± SD of serum tyrosine kinase levels across different groups. In the control group, the mean concentration of tyrosine kinase is 3.73 ng/mL, while untreated thyroid cancer (TC) patients exhibit a substantially elevated concentration of 17.79 ng/mL. Treated TC patients, on the other hand, demonstrate a mean concentration of 7.63 ng/mL. The observed p-value, < 0.0001, indicates a statistically significant difference in tyrosine kinase levels among the groups.

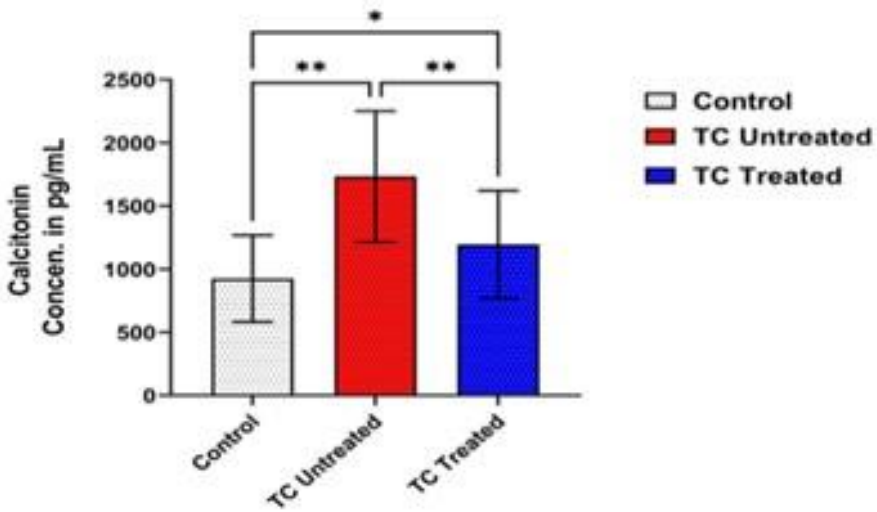

Figure 7. Tyrosine kinase enzyme concentration in study groups.
*single star indicates the presence of significant differences between the study parameters,
** double stars indicate the presence of high significant differences between the study parameters. at P value<0.0001

Outcomes regarding tyrosine kinase levels reveals notable differences among distinct groups where there was a noteworthy difference between TC Treated and TC Untreated with similar results between control and TC treated. In the control group, the mean concentration of tyrosine kinases stood at 4 ng/mL, whereas untreated thyroid cancer patients exhibited a significant analyzing the increase to 17 ng/mL. Conversely, treated thyroid cancer patients displayed a relatively lower concentration of 6 ng/mL. These results suggest a potential correlation between tyrosine kinase levels and the progression of thyroid cancer, with untreated patients showing the highest levels (see Figure7). These results lead to the findings that, the Tyrosine kinase levels can be a sign for TC disease. As mentioned in the study of [11] and [12].

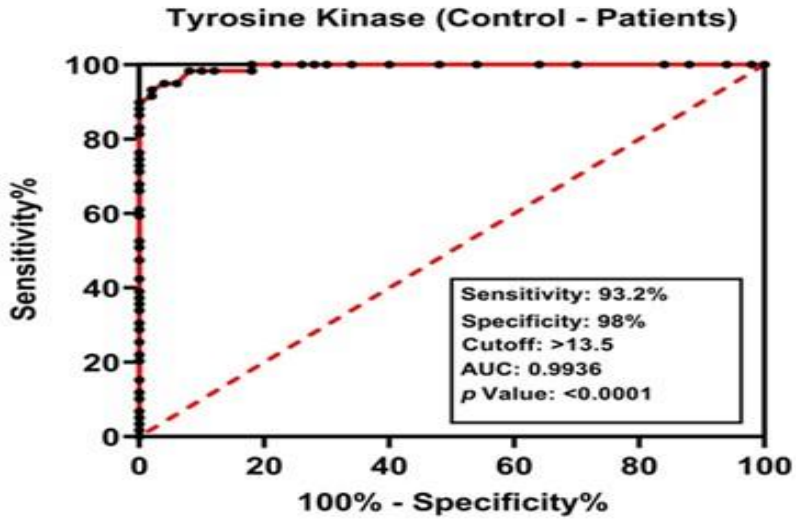

Figure 8. ROC Plot showing the summary curve of basal reported tyrosine kinase cut-off values.

Moving on to the Receiver Operating Characteristic Curve (ROC) analysis for tyrosine kinases, the results indicate a promising diagnostic potential. The analysis yielded a sensitivity of 93.2% and specificity of 98% at a cutoff value of >13.5 ng/mL. Additionally, the Area Under the Curve (AUC) was calculated to be 0.9936, demonstrating excellent discriminatory power between thyroid cancer cases and controls. The associated p-value, which was <0.0001, underscores the statistical significance of these findings (figure 8). The study results refer to two sides, first one that the calcitonin hormone high levels can be one of TC parameter as shown in figure (5) that control group have a low level of this hormone, while the untreated group show the highest level comparing with treatment group which have calcitonin level abroach control group level, the result agree with study done by [13] and [14]. Second one, that tyrosine kinase enzyme high values can also use as a sign for this disease as figure (7) exhibit that treated group show decreasing in tyrosine kinase level and abroach from control group, while untreated group exhibit high level in this enzyme many studies focused on the tyrosine kinase role in detecting TC, such as [15] and [16] that mean the chemical treatment inhibiting the development of TC.

4. Conclusions

In our study, the levels of calcitonin were measured and compared between the studies of three groups, and found that calcitonin high levels can be a sign for TC disease, also the levels of important enzyme tyrosine kinase was examined in TC patients. The enzymes exhibited substantial differences between TC with both of two groups of patients of TC (with treatment and untreated) and control groups, indicating that tyrosine kinase plays an important role as indicator for this disease. When correlation analysis are analyzed, the correlation of the parameters shows that enzyme and calcitonin play a role together in the disease. TC is an important disease that can be found in many people and seriously affects their life. Therefore, it is important to better elucidate the pathogenesis of the disease.

Conflicts of Interest: The authors confirm that there are no conflicts of interest.

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