**Original Research**

# Examination of C-Peptide Levels in Plasma at the Beginning of Type 1 Diabetes Diagnosis in Children during the Honeymoon Period

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**Abstract**

**Background:** C-peptide determination in the honeymoon phase is a practical approach to start or stop insulin-based treatment in patients suffering from type 1 diabetes (T1DM). The present study aimed to assess the C-peptide levels in plasma at the beginning of type 1 diabetes diagnosis in children during the honeymoon period.

**Methods:** Younger than 18 years old children with proven insulin-dependent diabetes who didn't receive any insulin treatment were included in the study. Those related to non-T1DM (T2DM, MODY, and secondary diabetes) and primary insulin administration, were excluded. Totally, 30 patients were entered. Blood samples were collected and C-peptide levels, fasting and random glucose, blood glycosylated hemoglobin (HbA1C), and serum levels of GAD (anti-glutamic acid decarboxylase antibodies), IA-2 (anti-tyrosine phosphatase antibodies), ICA (islet cell antibodies), and IAA (insulin autoantibodies) were analyzed.

**Results:** The age of patients had a range of 1 to 17 years old. The honeymoon period had a range between 3 to 18 months. Only 7 out of 30 (23.33%) studied patients had C-peptide levels of <5 before the honeymoon period. Reversely, 23 out of 30 (76.66%) patients had C-peptide levels of <5 after the honeymoon period. Additionally, IAA, ICA, GAD, and TA2 autoantibodies were detected in 23.33% (7 out of 30 patients), 20% (6 out of 30 patients), 66.66% (20 out of 30 patients), and 23.33% (7 out of 30 patients), respectively.

**Conclusion:** These findings may show the high impact and role of GAD autoantibody and also C-peptide during the honeymoon period.

**Keywords:** C-peptide, Determination, Type 1 diabetes, Children, Honeymoon Phase

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## Introduction

Type 1 diabetes mellitus (T1DM) is the predominant type of diabetes in children (1). In most cases, it is caused by the autoimmune destruction of pancreatic β-cells (insulin-producing cells) giving rise to insulin deficiency and subsequent hyperglycemia (2). The T1DM incidence has increased in children globally, and about 80,000 children below 15 years old develop T1DM yearly (3). As a result, T1DM requires more attention rendering its high morbidity, economic burden, and even mortality. The T1DM honeymoon phase is a short period after the start of insulin treatment when it seems that part of the pancreatic β-cells function is remaining (4). During this phase, patients need less insulin with better metabolic control of hypoglycemia. In some cases, patients can live without the exogenous insulin administration (5). It has been observed that only 50% of T1DM children enter the honeymoon period and those who do not enter the remission phase experience more short-term and long-term complications (6). The data from previous studies have shown that the recovery rate is between 25 and 100% and varies between 1 month and 13 years (7). In this period, the possibility of drug intervention may stop the destructive procedure of pancreatic β-cells, and subsequent insulin secretion (8). Several clinical and metabolic factors affect the frequency and duration of the honeymoon period. One of these is connecting peptide (C-peptide) (9). C-peptide is the part of proinsulin that is cleaved before co-secretion with insulin from pancreatic β-cells (10). In the other hand, C-peptide measurement in blood and urine has been used as a biomarker of pancreatic β-cells function because it is secreted in equimolar amounts with insulin and, unlike insulin, is not extracted by the liver (11). Therefore, the C-peptide test is used to track the pancreatic β-cells and help medical practitioners determine when to start insulin treatment (12). The present study was conducted to evaluate the level of C-peptide in serum and urine in T1DM patients at the time of diagnosis of diabetes and during the honeymoon period, to investigate the type of diabetes, the duration of the remission phase, the reduction of the need for insulin, and the treatment method.

## Methods

### Ethics

The present survey was ethically approved by the ethical council of the Zahedan University of Medical Sciences, Zahedan, Iran (Ethical code of 99-1-142-48252). Identical information of all pediatric patients was kept secret. Sampling was done in a way that the lowest pain faced to all pediatrics and also in a moral manner. The consent form was completed by the parents of the patients.

### Study criteria and patients

The current cross-sectional study was conducted on T1DM patients under 18 years old who were diagnosed at the Children's Medical Center Hospital and referred to the Endocrine Clinic Zahedan University of Medical Sciences, Zahedan, Iran.

According to ISPAD (13), fasting blood sugar (FBS) greater than or equal to 126, or blood sugar 2 h after a meal greater than or equal to 200, or random blood sugar greater than 200 with symptoms of polyuria and polydipsia, or HBA1C greater than or equal to 6.5%. were considered as diabetes and were included in the study.

### Inclusion and exclusion criteria

Pediatrics who passed the following criteria were included in the study:

1. Proven insulin-dependent diabetes
2. Age less than 18 years old
3. Insulin treatment has not started yet

Pediatrics who had any of the following criteria were excluded from the study:

1. Patients suspected of non-T1DM (T2DM, MODY, and secondary diabetes)
2. Unwillingness of the patient or their parents to enter or continue follow-up in the study
3. Primary treatment has started.

### Sampling

From all T1DM patients who met the criteria for entering the study, 5 ml of blood samples were collected using tubes containing EDTA.

Additionally, their urine samples were collected. Both samples were analyzed for the presence of C-peptide. During the follow-up, from the patients who entered the Honeymoon phase based on the criteria, blood and urine samples were collected again for C-peptide analysis.

**Study procedure**

The sample size was measured after determining the frequency of single nucleotide polymorphism in the Iranian population for Power>80%. Thirty pediatric patients with the above-mentioned criteria were included in the survey.

According to the latest ISPAD guidelines (13), the honeymoon period was determined based on the

**Following formula:**

Insulin dose adjusted HbA1c, defined as HbA1c (%)+4 × [insulin dose in U/Kg/24 h]<9

Because HBA1C may not be a good criterion in the early diagnosis of the disease, the daily insulin requirement of less than 0.5I U/Kg/day was considered as the honeymoon period under the condition of clinical control of the patient's sugar . The anthropometric, clinical, and biochemical findings of the patients were collected and included in the pre-prepared checklist.

**Biochemical analysis**

The blood sample was collected from the anterior cubital fossa (3 ml) following the standard procedures. Blood glucose was checked using a glucometer (Accu-Check Active- 50 Count, Germany). Blood glycosylated hemoglobin (HbA1C) was estimated according to turbidimetric analysis by spectrophotometer (Shimadzu, Japan). C-peptide was measured using an enzyme-linked immunosorbent assay (ELISA) (Modular Analytics E170, Roche Diagnostics, Singapore) rendering the kit guidelines. The serum levels of GAD (anti-glutamic acid decarboxylase antibodies), IA-2 (anti-tyrosine phosphatase antibodies), ICA (islet cell antibodies), and IAA (insulin autoantibodies) were analyzed using MAGLUMI 800 Chemiluminescence Immunoassay (CLIA), The procedure was performed following recommended protocols by the manufacturer. The positive cutoff values for GAD, ICA, IAA, and IA2 were 17 IU/mL, 28 U/mL, 20 IU/mL, and 28 IU/mL, respectively.

**Results**

**Demographical and clinical characters**

Table 1 shows the demographical and clinical characteristics of the studied population. Age of all examined patients was between 1 to 17 years old. The honeymoon period had a range between 3 to 18 months. C-peptide level of patients after the honeymoon period was <5 in majority of cases but it was borderline in majority of cases before the honeymoon stage. GAD autoantibody was more detected amongst the patients. And 80% patients c peptide level decreased in honeymoon and reached <5.

**Discussion**

T1DM is a metabolic disease with unknown causes that occurs as a result of a disorder in the immune system (14). In this disease, the body's immune system begins to destroy the pancreatic β-cells, and as a result, the body lacks the insulin hormone causing an increase in the amount of blood sugar (14). In almost 80% of people suffering from T1DM shortly after the insulin injection, a partial recovery is felt (15). Transient reduction in the required insulin, which is called the honeymoon phase, is when the pancreatic β-cells are still able to produce a small amount of insulin to reduce the need for insulin and help control blood sugar (16). During this phase, despite fluctuations in diet and physical activity, the blood glucose level is almost stable and within the normal range (17). The prevalence and duration of the honeymoon phase in T1DM patients are different and can last from one week to several years (18). Studies showed that C-peptide level determination in this phase can effectively determine the exact time to start insulin administration in T1DM patients (4, 5).

The present study aimed to assess the level of C-peptide in serum and urine in T1DM patients at the time of diagnosis of diabetes and during the honeymoon period. Findings showed that only 7 out of 30 (23.33%) studied patients had C-peptide

levels of <5 before the honeymoon period. Reversely, 23 out of 30 (76.66%) patients had C-peptide levels of <5 after the honeymoon period. Additionally, IAA, ICA, GAD, and TA2 autoantibodies were detected in 23.33% (7 out of 30 patients), 20% (6 out of 30 patients), 66.66% (20 out of 30 patients), and 23.33% (7 out of 30 patients), respectively. These findings may show the higher impact and role of GAD autoantibody in the T1DM at the honeymoon period.

A previous study (19) showed that about 57.80% of diabetic children under the age of 18 did not enter the remission phase at all, and diverse factors, such as serum bicarbonate less than 15 at the time of diagnosis, young age, increased body capacity, and female gender may affect this phenomenon. In the previous survey, two different methods were used to determine the honeymoon period (19). A C-peptide above 300 was considered as a remission period, but in younger infants, since the amount of C-peptide is lower, the ISPAD formula was used based on HBA1C and total serum dose. Additionally, because the amount of HBA1C is different based on the geographical region, each region should be examined separately (19). Rydzewska et al. (2019) (20) assessed 82 T1DM patients over 2 years. They showed that patients who had no physical activity before T1DM diagnosis, had more acid and base imbalances and the group with high physical activity needed less insulin with higher C-peptide levels and lower HBA1C (20).

Hwang et al. (2017) (21) in a Korean survey, divided the T1DM patients into two different groups based on the C-peptide level. Those who had C-peptide levels less than 0.6 ng/ml had lower age and BMI at the time of diagnosis and even harbored the diagnostic levels of C-peptide even after 36 months, which showed the high importance of C-peptide level analysis. Paying attention to the fact that when diabetes is diagnosed, insulin treatment should be started as soon as possible, which may leave more reserves of pancreatic β-cells. This will reduce the need for insulin, and reduce hypoglycemia and cardiovascular complications in the future. Jones et al. (2012) (22) showed that the pancreatic β-cells reserve can be determined using the C-peptide analysis. They suggested that because there may be an overlap between type 1 and type 2 diabetes in some cases of C-peptide analyzing, C-peptide should be measured long-term and sometimes 3-5 years later to distinguish type 1 from type 2 diabetes. From 3 to 5 years after the diagnosis, the possibility of type 2 diabetes is raised (23). Faustman and Davis (24) reported that C-peptide level analysis even decades after diabetes can be a good measure to predict complications and the remaining function of pancreatic β-cells. It has been accepted that the level of C-peptide greater than 10 is associated with a reduction in the complications of nephropathy, retinopathy, and neuropathy and should be part of the regular examination of diabetes (24).

The benefit of measuring C-peptide is more than that of insulin because it is not affected by exogenous insulin and C-peptide which is the insulin administered in the vein. The port is secreted and reflected and the peripheral insulin level may not be accurate. Additionally, the half-life of C-peptide is 5 times that of insulin. In a study on 182 patients (25), it was shown that those who had a higher C-peptide level had better control of blood sugar, and the serum C-peptide level in the range of 2.8 pmpl/l, the function of Langerhans islet cells remains intact. A study conducted in Iraq on 64 diabetic children (26) showed that those who enter the remission phase have a higher C-peptide level, and the level of 100-200 pmol/l is a good predictor. It is for the remission phase and this number was considered a cut-off.

**Conclusion**

In conclusion, the findings of this survey showed that C-peptide analysis can be an efficient method to find the exact procedure of T1DM and also insulin administration in patients. Only 23.33% of examined patients, had C-peptide levels of <5 before the honeymoon period. However, after the

honeymoon period, C-peptide was measurable in 76.66% of patients. Additionally, GAD was determined as the most frequent autoantibody amongst the T1DM patients in the honeymoon period.

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**Authors Contributions:**

MNM, ESS conceptualized the study objectives and design. MNM, ESS are infectious disease specialists who contributed to data collection from patients along with MNM, ESS drafted the study design protocols to be submitted to research centers. Data were was analyzed by MNM, ESS. Manuscript was drafted by MNM, ESS. All authors contributed in revisions.

**Ethical Consideration:**

IR.TUMS.CHMC.REC.1399.046

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**Tables:**

**Table 1. Demographical and clinical characters.**

| Pediatric No | Age (Year) | Honeymoon period (Month) | C-peptide level (ng/ml) | | Autoantibodies | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Before honeymoon | After honeymoon | IAA | ICA | GAD | TA2 |
| 1 | 10 | 7 | Borderline | Borderline | - | - | - | - |
| 2 | 7 | 12 | Borderline | <5 | - | - | + | - |
| 3 | 1 | 3 | Borderline | <5 | - | - | - | + |
| 4 | 5 | 16 | Borderline | <5 | - | - | + | - |
| 5 | 9 | 18 | Borderline | <5 | + | - | - | - |
| 6 | 4 | 8 | Borderline | <5 | - | + | + | - |
| 7 | 1.5 | 6 | Borderline | Borderline | - | - | - | + |
| 8 | 2 | 8 | <5 | <5 | - | - | + | - |
| 9 | 11 | 14 | Borderline | <5 | - | - | + | - |
| 10 | 2 | 15 | Borderline | <5 | - | + | + | - |
| 11 | 6 | 12 | <5 | <5 | - | - | + | - |
| 12 | 3 | 13 | <5 | <5 | + | - | + | - |
| 13 | 8 | 18 | Borderline | <5 | - | - | + | - |
| 14 | 5 | 10 | Borderline | <5 | - | - | - | + |
| 15 | 17 | 17 | Borderline | <5 | - | - | + | + |
| 16 | 2.5 | 15 | Borderline | Borderline | + | - | + | + |
| 17 | 3 | 12 | Borderline | Borderline | - | + | - | - |
| 18 | 11.5 | 15 | <5 | <5 | - | - | + | + |
| 19 | 3 | 7 | Borderline | <5 | - | + | - | - |
| 20 | 8 | 10 | <5 | <5 | + | - | + | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 21 | 13 | 14 | Borderline | Borderline | + | - | - | - |
| 22 | 2 | 16 | Borderline | <5 | - | + | + | - |
| 23 | 10 | 8 | Borderline | Borderline | - | + | + | - |
| 24 | 3 | 14 | Borderline | <5 | + | - | - | - |
| 25 | 15 | 12 | Borderline | Borderline | - | - | + | - |
| 26 | 15 | 12 | Borderline | <5 | + | - | + | - |
| 27 | 12 | 12 | Borderline | <5 | - | - | + | + |
| 28 | 11 | 8 | <5 | <5 | - | - | - | - |
| 29 | 9 | 10 | Borderline | <5 | - | - | + | - |
| 30 | 8 | 7.5 | <5 | <5 | - | - | + | - |