TJPS



Tikrit Journal of Pure Science

ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)



Evaluation the Iron status and some electrolytes balance in patients with chronic renal failure

Wissam Sabhan Khalaf¹, Wasan Nazhan Hussein¹, Amina Hamid Ahmed² ¹Department of Chemistry, College of Education for Pure Sciences, University of Tikrit, Tikrit, Iraq ²College of Veterinary Medicine, University of Kirkuk, Kirkuk, Iraq

https://doi.org/10.25130/tjps.v24i4.398

ARTICLE INFO.

Article history: -Received: 26 / 2 / 2019 -Accepted: 16 / 5 / 2019 -Available online: / / 2019 Keywords: iron level, kidney failure, electrolytes. Corresponding Author: Name: Wissam Sabhan Khalaf E-mail:

Wissam928218@yahoo.com Tel:

ABSTRACT

Background: In developing countries, chronic kidney disease (CKD) associated with anaemia is one of the major public health problems. With the progression of the disease, development of haematological abnormalities including iron deficiency increases. Renal anaemia may further increase the morbidity in these patients. Therefore, earlier detection and treatment of anaemia may be helpful in preventing the progression of the diseases and its other adverses (complications) outcomes.

Materials and Methods: The study included estimation of the level of iron , some electrolytes with the determination of effect of body mass on all biochemical variables in the serum of healthy people the number (40) sample and people with chronic renal failure the (90) sample.

Results: The Results indicate that the levels of serum iron ,Total ironbinding capacity (TIBC) ,Transferrin Saturation (T.S), haemoglobin , PCV, calcium and magnesium were Significantly Decreased in Patients group as compared with control and Significant Height in phosphate level (P \leq 0.01) in Patients group as compared .

Introduction

Chronic Renal Failure (CRF) is a global omit it health problem with a growing prevalence of disease [1]. The prevalence of CRF is estimated to be (8-16)% omit it one of the most prevalent worldwide diseases [2], especially in developing countries. The main cause of CRF is renal glomerulonephritis, although infection has become less it [3]. Current evidence suggests that diabetes and hypertension were the main causes for kidney disease[4]. In developing countries CRF is a major burden on patients to the extent that renal failure requires replacement therapy and is considered a difficult and costly treatment for patients[5]. However, less than 10% of stage end kidney patients have access to some form of substitution therapy College [6].

Anemia is one of the causes of iron deficiency common in patients with chronic renal failure, and in many cases iron deficiency is due to the low intake of food containing iron or vitamins or dysfunction in the process of formation of red blood cells in the bone marrow, The lack of iron leads to a decrease in the formation of hemoglobin, causing anemia. Other causes of anemia in patients with renal failure include hypothyroidism, cytokines, half-life of red blood cells, vitamin deficiencies (folate and vitamin B12) [7]. Anemia patients have been losten appetite, nausea, vomiting and eating low amounts of nutrients needed to be red blood cells[8], There dietary of protein intake in patients with renal failure play a role in anemia. Iron deficiency is also associated with Total Iron Binding Capacity (TIBC) and transferrin saturation (TS) [9], which is the ratio of serum iron associated with proteins to the formation of haemoglobin and iron level in patients Iron deficiency sufferers are less than (20%) causing anemia [10], (TS) is continuously and extensively altered as a result of the daily change in iron serum. where the present study was conducted to assess iron status in CRF patients, and Iron deficiency is common in patients [11-12].

The imbalance of calcium, phosphorus and magnesium leads to the recurrence of serious clinical complications including arrhythmias and difficulty in breathing. The kidney plays a major role in regulating the levels of ions and important elements in the serum and regulating the level of calcium, phosphate and magnesium occurring in different parts of the nephrons and any abnormalities in the kidney, weak control of the renal control of these ions leads to imbalance in the distribution of ions and their level have many physiological effects in patients. The level of ions is maintained by careful alterations of urine secretion and maintenance of normal levels [13].

Materials and methods

study design: This study was conducted on 130 sample of serum as a control and chronic kidney disease Patients , (male and female) with age ranged between (23-66) years , which was conducted at the Kirkuk General Hospital - Kidney dialysis Unit in the city of Kirkuk from the beginning of February 2018 to the end of September 2018.

The level of serum Iron and TIBC was determined by using Diagnostic kit ready (French company Biolabo) [14]. T.S was then calculated from the following equation [15].

transferrin saturation = TIBC x100%

The calcium, magnesium and phosphates level was determined using the colorimetric method a special work kit equipped by the (French company Biolabo) [16] [17].

Statistical Analysis

The results of the study were statistically analyzed using SPSS. The t-test was used to show the difference between two groups at a probable level (P <0.01) and the ANOVA test to show the difference among three groups.

Results and discussion

The results of the study included the statistical values of (Iron, TIBC, TS%, Hb, PCV) omit it some electrolytes (Ca, Mg, PO4) and measured in patients compared to the control group (healthy).

Iron concentration

In this study the mean \pm SD of serum Iron, Total Iron-Binding Capacity, Transferrin saturation where Summarized in table (1) and also Hemoglobin and Packed Cell Volume in the whole blood.

Table (1) The mean ±SD of Iron ,TIBC, T.S %,Hb , PCV for groups under investigation

| I C V for groups under investigation | | | | | |
|--------------------------------------|--------------------|--------------------|--------|--|--|
| Parameters | Control | Patients | P≤0.05 | | |
| | (Mean ±SD) | (Mean ±SD) | | | |
| Iron (µg\dl) | 102.60 ± 25.24 | 62.81 ± 15.90 | 0.0001 | | |
| TIBC (µg\dl) | 435.93 ± 61.59 | 305.80 ± 59.09 | 0.0001 | | |
| T.S (%) | 23.99 ± 5.85 | 21.13 ± 6.71 | 0.016 | | |
| Hb (g/l) | 12.75 ± 1.05 | 9.05 ± 0.75 | 0.0021 | | |
| PCV (%) | 40.80 ± 3.29 | 29.43 ± 2.58 | 0.0001 | | |

This result indicates that the level of Iron, TIBC, TS, Hb and PCV were significantly decreased in patients group as compared with control, this results was agreement with the result of (TIETZ) [18], where it was found that low concentration of iron leads to anemia which is the main feature of chronic renal failure. Iron deficiency affects more than 2 billion people all over the world and causes iron deficiency remains the leading cause of anemia, as it is now at the highest level in central and west Africa and south asia because patients with CKD, They have nutritional deficiencies and reduced intestinal absorption of iron and gastrointestinal bleeding may lead to anemia due to iron deficiency [19]. TIBC in the serum showed the strongest positive correlation with serum iron and the negative correlation with the serum ferritin concentration [20]. The positive correlation of TIBC with iron is evident in the study due to their low concentration of whose level was (T.S) together level was when the patients, $(21.1 \pm 6.7\%)$ compared with the healthy group, The saturation ratio was $(23.9 \pm 5.8\%)$ (usually very low) [20] In dialysis patients and the reason for this is the loss of iron during the process of washing and iron intake in the diet to adequately meet the need of the body because the washing process frequent This is consistent with the study [19] The level of iron and (TIBC), the percentage of saturation (T.S) are shown in the following figure.

The causes of the reduction of Hb and PCV in chronic renal failure patients refer to the lack of hormone Erythropoietin, which is responsible for the formation of red blood cells in the bone marrow, and the lack of red blood cells leads to a decrease in the concentration of hemoglobin and the percentage of compressed blood cells The epithelial cells surrounding the renal tubules in the formation of Erythropoietin and any disorder affecting these cells cause the reduction of the secretion of the hormone Erythropoietin [21], [22].

The results indicate a shortage of production the erythropoietin hormone because of the damage in the cells surrounding the renal tubules and thus lead to a decrease in the production of red blood cells from the bone marrow, which means the occurrence of Pertdam and the effectiveness of the hormone erythropoietin and its ability to bind receptors on the surface of cells generating red blood cells from Bone marrow which is affected by an the rise in increased thyroid hormone that is elevated in patients with renal failure. [22]

By studying the effect of body mass in Patients chronic renal failure on Iron Concentration, Total Iron Binding Capacity, Transferrin saturation, Hemoglobin, and Packed Cell Volume, as shown in the following table ;-

Table (2) Effect of body mass Index in the mean ±SD of Iron ,TIBC, T.S %,Hb , PCV for groups under

| investigation | | | | | |
|---------------|-----------------------------|----|---------------------------|--|--|
| Parameters | Group BMI kg\m ² | Ν | Patients Mean ±SD | | |
| Iron | G ₁ (16-20.9) | 28 | 63.1 ^a ± 14.7 | | |
| g/dlµ | G ₂ (21-24.9) | 51 | 62.4 ^a ± 17.8 | | |
| | G ₃ (25 - 30) | 11 | $61.0^{a} \pm 7.2$ | | |
| TIBC | G ₁ (16-20.9) | 28 | $299.7^{a} \pm 61.4$ | | |
| g/dlµ | G ₂ (21-24.9) | 51 | 311.5 ^a ± 59.3 | | |
| | G ₃ (25 - 30) | 11 | $289.4 ^{a} \pm 56.6$ | | |
| T.S (%) | G ₁ (16-20.9) | 28 | 21.9 ^a ± 6.6 | | |
| | G ₂ (21-24.9) | 51 | 20.3 ^a ± 6.9 | | |
| | G ₃ (25 - 30) | 11 | 21.9 ^a ± 5.6 | | |
| Hb | G ₁ (16-20.9) | 28 | 9.13 ^a ±0.91 | | |
| g/dl | G ₂ (21-24.9) | 51 | $9.02^{a} \pm 0.68$ | | |
| | G ₃ (25 - 30) | 11 | $9.06^{a} \pm 0.58$ | | |
| PCV (%) | G ₁ (16-20.9) | 28 | 29.53 ^a ± 3.15 | | |
| | G ₂ (21-24.9) | 51 | 29.45 ^a ± 2.26 | | |
| | G ₃ (25 - 30) | 11 | 29.63 ^a ± 1.91 | | |

In throughout of the effect of body mass on the iron group, there were no significant differences between

TJPS

the groups of patients and the mass of the body which did not affect the level of variables, where it was found that the level of iron did not change significantly at the groups of patients at the level of probability (P≤0.01) and the level of (TIBC) it was found that there were no significant differences in patients (P \leq 0.01), By calculating the saturation ratio (TS), it was found that there were no significant differences in patients by the effect of body mass as shown in the table, This is consistent with many studies (Akram et al) [23]. It shows that the level of iron and TIBC is no significantly affected by the mass of the body and also corresponds to the study of (Hea) [24] in that the iron is not significantly affected by the mass of the body while disappearing with the level of (TIBC) The following figure shows the level of body mass effect on the iron group.

Focusing on the effect of body mass on the hemoglobin and blood corpuscles found that there were no significant differences between the groups of patients and the mass of the body does not affect the level of variables It was noted that the level of hemoglobin did not change significantly in the groups of patients at the level of probability ($P \le 0.01$), The PCV level did not change significantly in the patient groups at the probability level ($P \le 0.01$) as shown in Table (3), This is consistent with several studies including (Olutavo et al) [25] . and show that the level of hemoglobin and blood-compressed blood cells are no significantly affected by the mass of the body and the following figure shows the effect of body mass level on hemoglobin and bloodcompressed blood groups among patients among them

Concentration of electrolytes

In this study, concentrations of some electrolytes were measured in the serum of patients with renal failure and control group , including calcium, magnesium and phosphate. The results of electrolyte levels were illustrated in Table (4)

Table (3) The mean ±SD of electrolytes for groups

| under investigation | | | | | | |
|---------------------|---------------|-----------------|---------|--|--|--|
| Parameters | Control | Patients | P≤0.01 | | | |
| (mg / dl) | Mean \pm SD | Mean \pm SD | T- test | | | |
| Calcium | 9.57±1.22 | 6.83 ± 1.37 | 0.0013 | | | |
| Magnesium | 2.40 ± 0.25 | 1.40 ± 0.34 | 0.0037 | | | |
| Phosphate | 3.72 ± 0.86 | 7.02 ± 0.27 | 0.0021 | | | |

This result indicate that the level of Ca , Mg , were significant decreased and increase significant PO_4 in patients group as compared with control .

The results showed a decrease in the level of calcium in patients was $(6.8 \pm 1.3 \text{ mg} / \text{dl})$ compared with the group of control $(9.6 \pm 1.2 \text{ mg} / \text{dl})$ and found that there were significant differences at the level of probability (P ≤ 0.01), In the level of calcium in the serum of chronic renal deficit patients compared to the level of calcium in the serum control group and this is consistent with the results of researchers[26], who indicated that there is a decrease in the level of calcium in the serum of patients with renal

impairment and may be attributed to the increase level Phosphorus The level of phosphate was patient group (7.02 \pm 0.2 mg / dl) compared with the control group $(3.7 \pm 0.8 \text{ mg} / \text{dl})$ and there was a significant difference at the probability level(P≤0.01), In the level of phosphates in the serum of patients with chronic renal disability, there is an inverse correlation between phosphorus and calcium, as they maintain the balance of concentration in the body as in the case of increasing the concentration of one of the concentration of the other decreases and may decrease the level of calcium due to a disorder in the metabolism of vitamin D, In patients with chronic renal disability [27] because of the inability of the kidney to form an effective form of vitamin D, which is necessary in the process of absorption of calcium, so the lack of vitamin D function causes a decrease in absorption of calcium from the intestine [28].

Results showed that the concentration of magnesium in patients $(1.40 \pm 0.34 \text{ mg} / \text{dl})$ compared with the healthy level $(2.4 \pm 0.25 \text{ mg} / \text{dl})$ and found that there were significant differences at the level of probability $(p \le 0.01)$ In the level low calcium and magnesium and high phosphate due to this imbalance to reduce the effectiveness and ability of the kidney to work and put a proportion of proteins with the generosity, as is known, these proteins are associated with many elements and the treatment of some therapeutic drugs that reduce absorption Some elements are by the intestines [28] and their levels are shown in Figure (7) following

Study of the effect of body mass index on the level of calcium (Ca), magnesium (Mg) and phosphate (PO4) in patients with chronic renal failure compared to the results were as shown in the following table.

| for some electrolytes groups under investigation. | | | | |
|---|--------------------------|----|---------------------|--|
| Parameters | Group BMI | Ν | Patients | |
| (mg / dl) | kg\m ² | | Mean ±SD | |
| Calcium | G ₁ (16-20.9) | 28 | $6.83^{a} \pm 1.66$ | |
| | G ₂ (21-24.9) | 51 | $6.69^{a} \pm 1.19$ | |
| | G ₃ (25 - 30) | 11 | $7.40^{a} \pm 1.23$ | |
| Magnesium | G ₁ (16-20.9) | 28 | $1.33^{a} \pm 0.62$ | |
| | G ₂ (21-24.9) | 51 | $1.28^{a} \pm 0.57$ | |
| | G ₃ (25 - 30) | 11 | $1.21^{a} \pm 0.41$ | |
| Phosphate | G ₁ (16-20.9) | 28 | $7.04^{a} \pm 0.23$ | |
| | G ₂ (21-24.9) | 51 | $7.00^{a} \pm 0.27$ | |
| | G ₃ (25 - 30) | 11 | $6.97^{a} \pm 0.36$ | |
| | | | | |

Table (4) Effect of body mass Index in the mean ±SD for some electrolytes groups under investigation.

A study of the effect of BMI on electrolytes in the groups of patients with renal failure showed that there were no significant differences between the divided groups where the level of calcium in the first group ($6.8 \pm 1.6 \text{ mg} / \text{dl}$) The second group of patients was ($6.6 \pm 1.2 \text{ mg} / \text{dl}$) (in the third group it was ($7.4 \pm 1.2 \text{ mg} / \text{dl}$). This is consistent with many studies [29], The level of phosphate in the first group was ($7.0 \pm 0.2 \text{ mg} / \text{dl}$) while in the second group it was ($7.0 \pm 0.27 \text{ mg} / \text{dl}$) in the third group it was ($6.9 \pm 0.36 \text{ mg/dl}$) this results was agreement with the result of (Natalia) [30], The magnesium level was In the first group, ($1.69 \pm 0.2 \text{ mg} / \text{dl}$), whereas in the second

group it was $(1.72 \pm 0.3 \text{mg/dl})$. In the third group it was $(1.41 \pm 0.23 \text{mg} / \text{dl})$ this results was agreement with the result of (Lutfi)[29] where the level of electrolytes does not give a significant difference between groups of patients divided by body mass and the following figure shows the effect of body mass on the electrolytes in patients

References

[1] Dewardener HE. (1986). An outline of normal and abnormal function in the kidney. 4th edition Churchill Livingstone. New York:181-235.

[2] Jha V, Garcia GG, Iseki K, Zuo L, Naicker S, Plattner B, *et al.* (2013). Chronic kidney disease: global dimension and perspectives. 382(9888):260-72.

[3] Barsoum RS.(2006) Chronic kidney disease in the developing world. *N Engl J Med.* 354; 991-997.

[4] Charmaine EL, Matthew JO, Deanna MR, Janet EH. (2004) The growing volume of diabetes- related dialysis: a population based study. Nephrology Dialysis Trans plantation .19;3098-3103.

[5] Rajapurkar MM, John GT, Kirpalani AL, Abraham G, Agarwal SK, Almeida AF, et al .(2012). What do we know about chronic kidney disease in India: first report of the Indian CKD registry. *BMC Nephrol*.;13:10.

[6] Agarwal SK, Srivastava RK. (2009) Chronic kidney disease in India: challenges and solutions. *Nephron Clin Pract.* 111;197-203.

[7] Mehdi U, Toto RD. (2009) Anaemia, diabetes, and chronic kidney disease. Diabetes Care. 32(7):1320-6.

[8] Sweny P, Farrington K, Moorhead JF. (1989) Chronic renal failure in the kidney and its disorders. 9th editio. New Delhi, *India. Jaypee Medical.* 359-369.

[9] Brugnara C. (2003). Iron deficiency and erythropoiesis: new diagnostic approaches. *Clin Chem.* 49 ; 1573-1578.

[10] Wish JB. (2006). Assessing iron status: beyond serum ferritin and transferrin saturation. *Clin J Am Soc Nephro l.*(1) :4-8.

[11] Madhusnata D, Halder A, Podder S, Sen R, Chakrabarty S, Sengupta B, *et al.* (2006) Anemia and hemoglobinopathies in tribal population of Easrtern and North-eastern India. *Hematology*,11(5):371-376.

[12] Mohanty D, Gorakshakar AC, Roshan B, Colah R, Patel Z, Dilip C, *et al.*(2014). Interaction of iron deficiency anaemia and hemoglobinopathies among college students and pregnant women: a multi-center evaluation in india .38(4):252-257.

[13] Judith Blaine. (2015). Michel Chonchol and Moshe Levi Renal Control of Calcium, Phosphate, and Magnesium Homeostasis. *Clin J Am Soc Nephrol.* 10; 1257–1272,.

[14] Young D.S. (1995),Effect of Drugs on Clinical Laboratory Tests . 4th Ed p.361-364.

[15] Tietz. N.W. (2007). Clinical Guide to Laboratory Test .4th Ed. p.1062 – 1065

Conclusion

Low iron level, total iron-binding capacity, Transferrin saturation, hemoglobin, Packed Cell Volume, calcium and magnesium, and high levels of phosphate, and no effect body mass on level all variables

[16] Konieczny, A. and Ausubel, F.M.A. (1993). procedure for mapping Arabidopsis mutations using co-dominant ecotype-specific PCR-based markers". *Plant* J. 4 : 403–410.

[17] Burtis, E.R.(1995) Scandinavian Journal of Clinical and laboratory Tests.191-306.

[18] Tietz N.W. Ashwood, W. B. Saunders. (1999) . Text book of clinical chemistry,3rd Ed.CA. p.1245-1250.

[19] Rumi Deori1. (2016). Bedanta Bhuyan, Iron status in chronic kidney disease patients, International Journal of Research in Medical Sciences. Aug; 4(8): 3229-3234.

[20] Rachelle Bross, Jennifer Zitterkoph . Juhi Pithia . Deborah Benner, Mehdi Rambod. Csaba P. Kovesdy. (2009). Association of Serum Total Iron-Binding Capacity and Its Changes Over Time with Nutritional and Clinical Outcomes in Hemodialysis Patients, *Am J Nephrol.* 29:571–581.

[21] Al-Bayati, Alaa Mohammed Hamid. (2016). studied some immunological and bio chemical indicators in patients Renal Failure in Diyala Governorate, Master Thesis,.

[22] Manahil. Z. Ahmed ; I. M. T. Fadlalla ; Amel. O. Bakheit .(2018). Association of Uric Acid, Urea and Creatinine with Body Mass Index, Age and Gender . *December* . 1858-6716 .

[23] Akram Ghadiri-Anari, Narjes Nazemian, and Hassan-Ali Vahedian-Ardakani. (2014) . Association of Body Mass Index with Hemoglobin Concentration and Iron Parameters in Iranian Population.p. 3.

[24] Hea Shoon Lee .(2017). Comparison of Serum Iron, Total Iron Binding Capacity and hemoglobin A1c level according to Obesity in South Korean Adult . *International Journal of Applied Engineering Research* ; p15830-15837 .

[25] Olutayo Ifedayo Ajayi1, David Bolaji Akinbo and Adaobi Mary-Joy Okafor (2017). Correlation between Body Mass Index and Hematological Indices in Young Adult Nigerians with Different Hemoglobin Genotypes. *American Journal of Biomedical Sciences* . p: (5)

[26]. Bro S. (2003). "How abnormal calcium, phosphate and parathyroid hormone relate to cardiovascular disease". *Nephrol. Nurs.* J; 30 (3): 275-283.

[27] Kaplan L.A., Amadeo J. Pesce and Steven C. K. (2003), "Methods in clinical chemistry". 4th ed. *Mosby* – *U.S.A.* p 113.

[28] Levine C. and Colburn J.W.(1984). "Magnesium the mimiclantagonist of calcium". *N. Engl. J. Med.* 19:1253-1254



[29] Lutfi, Mohamed Faisal . (2014). Effects of age, body mass index and electrolytes levels on blood pressures of normotensive adults. *Khartoum Medical Juornal* . 5 : 673 - 681 . [30] Natalia Campos-Obando, W Nadia H Koek, Elizabeth R Hooker, Bram CJ van der Eerden, Huibert A Pols, Albert Hofman, atel . (2017). Serum Phosphate Is Associated With Fracture Risk. Journal of Bone and Mineral Research . p 1182–1193.

تقييم حالة الحديد وتوازن بعض الالكتروليتات في المرضى الذين يعانون من الفشل الكلوي المزمن وسيم حالة الحديد وتوازن بعض الالكتروليتات في المرضى الذين عانون من الفشل الكلوي المزمن

¹ قسم الكيمياء ، كلية التربية للعلوم الصرفة ، جامعة تكريت ، تكريت ، العراق ²كلية الطب البيطري ، جامعة كركوك ، كركوك ، العراق

الملخص

الخلفية: في الكثير من البلدان وخاصة النامية منها، يعتبر مرض الفشل الكلوي المزمن (CRF) مرتبط ارنباط رئيسي بمرض بفقر الدم وهو عامل رئيسي لحدوث وتطور مشاكل الصحة العامة في الجسم مع تطور مرض الفشل الكلوي وحدوث تشوهات في الدم بما في ذلك نقص مستوى الحديدفي الجسم، ومن الممكن ان يزيد مرض فقر الدم الكلوي من تطور المرض لدى المرضى وفي وقت سابق كشفت الدراسات ان علاج فقر الدم قد يكون مفيد في منع تطور الأمراض وعدم حدوث تغير في مستوى مواد الجسم، الكالسيوم والفوسفات والمغنيسيوم هي الكتروليتات عبارة عن كاتيونات متعددة التكافؤ وتعتبر مهمة للكثير من الكائنات الحية والوضائف الخلوية وللكلية دورًا كبير في الحفاظ على هذه الأيونات ضمن مستواها الطبيعي.

المواد و طرق العمل: أجريت هذه الدراسة على 130 عينة من مصل دم مرضى الفشل الكلوي ومجموعة السيطرة مكونه من (نكور وإناث) تتراوح أعمارهم بين (23–66) سنة، والتي أجريت في مستشفى كركوك العام – وحدة غسل الكلى في مدينة كركوك من بداية فبراير 2018 إلى نهاية سبتمبر 2018.

النتائج: تبين من النتائج أن مستويات الحديد والسعة الكلية لربط الحديد (TIBC) و ونسبة تشبع النواقل (TS) والهيموغلوبين و وحجم كريات الدم الحمر والكالسيوم والمغنيسيوم في المصل انخفضت بشكل ملحوظ في مجموعة المرضى بالمقارنة مع السيطرة وارتفاع ملحوظ لمستوى الفوسفات في المصل عند مستوى الاحتمالية (0.01≤P) .