Comparison Study of the Effect of Erlotinib as a Tyrosine Kinase Inhibitor on Electrolyte Levels in Type2 Diabetic and Diabetic Nephropathy

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Abstract

Diabetes mellitus can be defined as a metabolic disorder disease. Complication of diabetes are due to diabetic nephropathy. This study was done ***in vitro*** to study the effect of different concentrations of erlotinib inhibitor (tyrosine kinase inhibitor) on electrolyte levels (Mg^{+2}, Ca^{+2}, Na^{+}) in sera of Iraqi patients with newly diagnosis type2 diabetes and diabetic nephropathy in addition to find the best percentage inhibition for utilizing different concentrations from erlotinib ($6.97x10^{-7}$, $9.30x10^{-7}$,$1.16x10^{-6}$,$1.39x10^{-6}$,$1.62x10^{-5}$) molar on electrolyte levels. This study was conducted in The National Diabetes Center, Al-Mustansiriya University - Baghdad and included 150 patients divided into50 patients type 2 diabetic as group (G2), 50patients diabetic nephropathy as group(G3) and also 50 healthy as control group(G1). The period time for aggregation the blood sampling was from July to October 2017. All patients were within (18 to 60) year's age. Erlotinib (Tyrosine kinase inhibitor) affected on serum Mg^{+2}levels in human as a mild effect and a slight effect on serum Na^{+} and Ca^{+2}.The best inhibition of erlotinib in concentration ($1.62x10^{-}5$) M for both serum Na^{+} and Ca^{+2} in newly diagnosis diabetes type 2 and diabetic nephropathy. Serum Mg^{+2}levels showed best inhibition in concentration ($9.30x10^{-}7$) M.

Keywords: Diabetes mellitus type 2, diabetic nephropathy, electrolyte (Mg^{+2}, Ca^{+2}, Na^{+}), erlotinib inhibitor.

1. Introduction

Diabetes mellitus can be defined as a metabolic disorder disease categorized by hyperglycemia resulting from defects in insulin secretion, insulin action or both [1]. Signs of hyperglycemia often contain polyuria, polydipsia, weight loss, polyphagia, and unclear vision [2]. Chronic symptoms of diabetes include micro vascular and macro vascular complications that lead to visual damage, blindness, kidney bug, nerve loss, amputation, heart illness, and stroke [3], and this disease affect a wide spread countries and in the Third World, about of 382 million people with diabetes in 2013, expected to rise to 592 million by 2035(4) The classification and diagnosis of diabetes established by the National Diabetes Data Group (NDDG)were published in 1979 [5]. Diabetes complication Diabetic Nephropathy (DN) is one of the most important long-term complication regarding morbidity and mortality in diabetics. The clinical syndrome of this disease is recognized by continual albuminuria, developmental decreased in the glomerular filtration rate and increased arterial blood pressure [6]. Erlotinib hydrochloride is an orally administered small molecule inhibitor of epidermal

growth factor receptor (EGFR) tyrosine kinase [7] and this drug a is quinazolinamine with the chemical name N- (3- ethynylphenyl)- 6, 7-bis (2- methoxyethoxy)- 4 - quinazolinamine [8]. Electrolytes are the chemical compounds present in body fluid and take part in some of the important body processes [9]. Disturbances in serum electrolyte levels are found to be associated with diabetes mellitus [10] Electrolyte derangement resulting from acute or chronic complications of diabetes [11].

2. Material and Method

This study was conducted in The National Diabetes Center, Al-Mustansiriya University – Baghdad. The results included 150 patients divided into50 patients with type 2 diabetic as (G2), 50 patients with diabetic nephropathy as (G3) and 50 healthy as control (G1), the period time for aggregation the blood sampling July to October 2017. All subjects were within (18 to 60) years age. Approximately (3) ml of venous blood was put in plan tube to evaluate serum Mg, Ca and Na and by centrifugation at 3500 rpm for 5 minutes. Then storage the serum in the freezing -20C° to the measured serum electrolyte before and after addition of erlotinib inhibitor. Serum magnesium was determined according to the manufacturer instruction as supplied with kit from Human Germany, Serum calcium was determined according to the manufacturer instruction as supplied with kit from Switzerland and Serum sodium was determined according to the manufacturer instruction as supplied with kit from Human Germany. Last different concentration was prepared from erlotinib inhibitor via weight (0.0075, 0.01, 0.0125, 0.015, 0.0175) mg and dissolved in distilled water and conversion to five concentration from erlotinib hydrochloride ($6.97x10^{-7}$, $9.30x10^{-7}$, $1.16x10^{-6}$, $1.39x10^{-6}$, $1.62x10^{-5}$) Molar , respectively [12].

2.1 Statistical Analysis

Results were expressed as Mean± SD. Statistical analysis was done by ANOVA to compare between three studied groups and unpaired student T-test to compare between two groups variation which are considered significant when P- values are ≤ 0.05.

3. Result and Discussion

3.1. A-Before Addition Inhibitor:

ANOVA test showed a highly significant difference for levels of serum (Mg^{+2},$Ca^{+2}$and Na^{+})when compared between three studied groups as shown in **Table 1**. Also in **Table 1.** explained the levels of serumMg^{+2}in G1 and G2 were (2.84± 0.34 and 2.87±0.24) mg/dL respectively ,and there was non-significant difference between two groups. These results are in agreement with previous studies [13-15] due to its diet low in magnesium, osmotic diuresis causing high renal excretion of magnesium [16].While the levels of serum Mg^{+2}in G3 and G1 were (2.38±0.08 and 2.87±0.24) mg/dl respectively, there was significant difference between two groups, due to Poor dietary intake, impaired absorption of magnesium, increased urinary loss because hyperglycemia, osmotic diuresis, defective Mg reabsorption from renal tubules and loss of plasma protein bound Mg [17]. These results are in agreement with previous study [15]. Also the levels of serum Mg^{+2} in G3 and G2 there were (2.38±0.08 and 2.84± 0.34) mg/dL respectively, there was significant difference between two groups. **Table 1.** explained the levels of serum Ca^{+2} in G2 and G1 there were (8.8±0.37 and 8.81±0.34) mg/dl respectively, there was non- significant difference between two groups. These result sare in agreement with previous study (18) due to the patients in G2 were newly diagnosed so that

the disorder in electrolyte did not begin yet , while the levels of serum Ca^{+2} in G3 and G1there were (9.01±0.5 and 8.81±0.34) mg/dl respectively, there was significant difference between two groups ,but the levels of serum Ca^{+2} in G3 and G2 were (9.01±0.5vs.8.8±0.37) mg/dl respectively, there was significant difference between two groups. The reason for elevated serum Ca^{+2} levels was explained Ca^{2+} reabsorption in proximal tubule was related with Na^{+} re absorption as well as Ca^{2+} competes with Mg^{2+} that transport in the loop of Henley [18]. depend on the Resnick ionic study suggested disorders like metabolic syndrome, hypertension and diabetes contribute to common intracellular condition in which decreased level of Mg^{2+} is associated with elevated free intracellular Ca^{2+} level [11]. **Table 1.** showed decrease significant difference in serum Na^{+} levels between G2vs.G1 (135.02± 1.99vs.138.68 ±1.35) mmo L/L respectively, due to hyperglycemia in diabetes lead to shifting water from intracellular space to extracellular space diluting the extracellular Na^{+} leading to lower serum Na^{+} level [19]. Alteration in rennin angiotensin system in diabetes leads to change in serum sodium concentration [20]. In the current study, the levels of Na^{+} in G1, G2 and G3 were (138.68± 1.35, 135.02 ±1.99 and146.22±29.26 mmol /L respectively, also there was a highly significant difference when compared between G3 with G1. These findings suggest kidney damage in individuals and this is in agreement with study [21].

3.2. B-After Addition Inhibitor

The recent study is considered as a first study *in vitro* for human that deals with the effect of different concentrations of erlotinib inhibitor on Mg^{+2} levels in diabetes type2 and diabetic nephropathy, so no clinical reports on the Mg^{2+} status after addition erlotinib inhibitor was available (22). The results in **Tables (2-6)** explain Mg^{+2} levels which show decrease of significant G2 when compared between before and after addition of five concentrations of erlotinib. The previous studies explain in mice that only a mild decrease in Mg^{2+} level was observed after addition of erlotinib, while the fractional excretion of Mg^{2+} remained unchanged (13). In G3, the Mg^{+2}levels show non-significant difference in concentration ($6.97x10^{-7}$ and $9.30x10^{-7}$) M, while they show significant difference in concentration ($1.16x10^{-6}$,$1.39x10^{-6}$ and $1.62x10^{-5}$) M, because the EGFR play a minimal biolovital role in control Mg^{2+} level at the nephron, so that the high dose from inhibitor leads to nephrons to preserve Mg^{2+} and causes moderate increase from Mg^{2+}level (12). The current study shows that the best inhibition in Mg^{2+}level in G2 at erlotinib concentration ($9.30x10^{-7}$) M, while in G3 best inhibition in Mg^{2+}level appeared at concentration ($1.62x10^{-5}$) M. The results of Ca^{+2}levels showed non-significant inhibition in Ca^{+2}levels in G2at concentration ($6.97x10^{-7}$, $9.30x10^{-7}$, $1.39x10^{-6}$, $1.62x10^{-5}$M), while in concentration ($1.16x10^{-6}$M) appeared decrease significant suggested the patients in G2 were newly diagnoses with diabetes mellitus and the disorder on calcium concentration did not start. There is no clinical study demonstrated the effect of erlotinib on calcium levels and this study is considered the first in human. In group G3 the Ca^{+2}levels show decrease of significant difference for all five concentrations of inhibitor. Demik in his study about mice is in agreement with the recent study that he observed about the systematic and renal Ca^{+2} homeostasis that still unaffected during adsorption of erlotinib because EGF doesn't directly influence Ca^{2+} handling so that the changes in renal Mg^{2+} concentration lead to subsequently correlate well with decrease fraction of distal tubular transport where Mg^{2+} transport is mechanistically separated from that of Ca^{2+}(12). In table (7) the best inhibition appeared in concentration (1.62×10^{-5}) M for G2 and

G3. At last the Na^+ levels show in **Tables (2-6)** the decrease of significant difference in concentration ($6.97x10^{-7}$, $1.16x10^{-6}$, $1.39x10^{-6}$, $1.62x10^{-5}$) M, while in concentration ($9.30x10^{-7}$) M appeared non-significant in G2. In group G3the Na^+ levels showed significant difference for five concentrations of erlotinib. These results are in agreement with study for another inhibitor Gefitinib (23), (24), and in agreement with the pervious study (25) that suggest in patients with T2DM, canagliflozin was generally associated with small mean percent changes in serum electrolytes (for both sodium and calcium concentration). In **Table 7.** the best inhibition in Na^+ levels in erlotinib concentration ($1.62x10^{-5}$) M for G2 and G3.

Table 1. Levels of S. (Mg^{+2}, Ca^{+2}, Na^+) of the studied groups before addition of inhibitor.

Parameters	G1(N =50) Mean ±SD	G2(N=50) Mean ±SD	G3(N=50) Mean ±SD	P-value	G2vs.G1	G3vs.G1	G3vs. G2
Mg^{+2}(mg/dL)	2.87±0.24	2.84±0.34	2.38±0.08	HS	NS	S	S↓
Ca^{+2}(mg/dL)	8.81±0.34	8.8±0.37	9.01±0.5	HS	NS	S↑	S↑
Na^+(mmol/L)	138.68±1.35	135.02 ±1.99	146.22±29.26	HS	S↓	HS↑	HS↑

Table 2. Levels of S. (Mg^{+2}, Ca^{+2}, Na^+) in concentration($6.97x10^{-7}$) M erlotinib.

Parameters	G2 No. (50) before	G2 No. (50) After	P value	G3 No. (50) before	G3 No. (50) After	P value
Mg^{+2}	2.84±0.34	2.59±0.51	S↓	2.38±0.08	2.35±0.04	NS
Ca^{+2}	8.8±0.37	8.65±0.1	NS	9.01±0.5	8.85±0.03	S
Na^+	135.02±1.99	135.87±4.45	S↑	146.22±29.26	156.73±1.17	S↑

Table 3. Levels of S. (Mg^{+2}, Ca^{+2}, Na^+) in concentration ($9.30\ x10^{-7}$M) erlotinib.

Parameters	G2 No. (50) before	G2 No. (50) After	P-value	G3 No. (50) before	G3 No. (50) After	P-value
Mg^{+2}	2.84±0.34	2 ±0.14	S↓	2.38±0.08	2.25±0.03	NS
Ca^{+2}	8.8±0.37	8.69±0.09	NS	9.01±0.5	8.68±0.04	S
Na^+	135.02±1.99	135.28±0.3	NS	146.22±29.26	145.62 ±0.68	S↑

Table 4. Levels of S. (Mg^{+2}, Ca^{+2}, Na^+) in concentration ($1.16\ x10^{-6}$M) erlotinib.

Parameters	G2 No. (50) before	G2 No. (50) After	P value	G3 No. (50) before	G3 No. (50) After	P value
Mg^{+2}	2.84±0.34	2.24±0.09	S↓	2.38±0.08	2.14±0.03	S
Ca^{+2}	8.8±0.37	8.55±0.11	S↓	9.01±0.5	8.55±0.03	S
Na^+	135.02±1.99	134.79±1.66	S	146.22±29.26	144.62±0.29	S↑

Table 5. Levels of S. (Mg^{+2}, Ca^{+2}, Na^{+}) in concentration (1.39×10^{-6}M) erlotinib.

Parameters	G2 No. (50) before	G2 No. (50) After	P value	G3 No. (50) before	G3 No. (50) After	P value
Mg^{+2}	2.84±0.34	2.26±0.23	S↓	2.38±0.08	1.95±0.04	S
Ca^{+2}	8.8±0.37	8.6±0.2	NS	9.01±0.5	8.45±0.03	S
Na^{+}	135.02±1.99	133.67±0.69	S	146.22±29.26	143.04±0.34	S↑

Table 6. Levels of S. (Mg^{+2}, Ca^{+2}, Na^{+}) in concentration (1.62×10^{-5}M) erlotinib.

Parameters	G2 No. (50) before	G2 No. (50) After	P value	G3 No. (50) before	G3 No. (50) After	P value
Mg^{+2}	2.84±0.34	2.08±0.13	S↓	2.38±0.08	1.91 ±0.03	S
Ca^{+2}	8.8±0.37	8.51±0.25	NS	9.01±0.5	8.34±0.03	S
Na^{+}	135.02±1.99	133.04±1.2	S	146.22±29.26	140.92±0.77	S↑

Table 7. The percentage inhibition of S. (Mg^{+2}, Ca^{+2}, Na^{+}) in G2 and G3

Concentration of erlotinib	G2 % Mg^{+2}	G3% Mg^{+2}	G2 % Ca^{+2}	G3% Ca^{+2}	G2 % Na^{+}	G3% Na^{+}
6.96×10^{-7}M	8.9 %	1.3%	1.8 %	1.8%	0%	0%
9.30×10^{-7}M	29.6 %	5.5%	1.3 %	3.7%	0%	0.5%
1.16×10^{-6}M	21.2%	10.1%	2.9%	5.2%	0.2%	1.1%
1.39×10^{-6}M	26.8%	18.1%	2.3%	6.3%	1%	2.2%
1.62×10^{-5}M	26.7%	19.8%	3.3%	7.5%	1.5%	3.63%

4. Conclusion

This study is considered the first study in Iraq that concluded the best percentage inhibition from utilizing different concentrations of erlotinib drug on electrolyte levels in type 2 diabetic and diabetic nephropathy. Therefore, a mild effect of erlotinib inhibitor on serum Mg $^{+2}$levels in human and a slight effect of erlotinib inhibitor on serum Na^{+} and Ca^{+2}.the best inhibition of erlotinib in concentration ($1.62 \times 10^{-}5$M) for both serum Na^{+} and Ca^{+2}, except Mg $^{+2}$levels showed best inhibition in ($9.30 \times 10^{-}7$M).

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