Determination of concentration intra-erythrocyte of metformin in type 2 diabetic patients with or without renal impairment : Comparative study of concentrations intra-erythrocytes between two groups of diabetic patients

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ABSTRACT : Type 2 diabetes and renal failure are insidious diseases. Worldwide, diabetes is the leading cause of chronic kidney disease. In Morocco, diabetes and kidney disease are two diseases on the rise and represent a real public health problem. The prevalence of diabetes in Morocco reached 9% of people aged over 20 years, 50% remain undiagnosed. Diabetes is the leading cause of chronic kidney disease whose prevalence is 2.9% in Morocco. The pathophysiology of type 2 diabetes appears to be dominated by insulin resistance and a lack of insulin secretion. Its management is based on the management of these two factors diabetogenic by physical activity, lifestyle changes and drug therapy (biguanides, glitazones, sulfonylureas). Metformin (biguanide) discovery in the 1950s is the oral antidiabetic medication, insulin sensitizer most prescribed in the world. In Morocco, it remains the most accessible for free. Its use requires compliance with its various cons-indications including impaired renal function. Metformin is excreted by the kidney with a clearance 440-450 ml / min. The main risk in renal insufficiency is lactic acidosis (LA) by accumulation or Intoxication with metformin. The intra-erythrocyte concentration of metformin is the best assessment of this accumulation indicator. The use of metformin may be as beneficial in patients with diabetes type 2 insulin-resistant renal impairment. Very few studies have been done on metformin and renal failure, hence the interest of our study in Morocco, Mediterranean region, to identify and study the difference of concentrations of metformin for type 2 diabetic patients with or without renal impairment.

Patients and Methods : We collected all the assay results of the intra-erythrocyte metformin during the period January 2013 and December 2014, including 64 type 2 diabetic patients on metformin time with their consent, and seen for the first in the Endocrinology service, Diabetology and Metabolic Diseases of the University Hospital (UH) Hassan II of Fez in Morocco for several reasons (imbalance of diabetes, therapeutic education, surgeries) . The creatinine clearance was estimated using the simplified MDRD formula (Modification of the Diet in Renal Disease). Statistical analysis was performed by Epi Info Version 7. The significance threshold is considered positive if p is less than 0.05.

Results : Among 64 identified patients (28 M / 36 W), 40.62% of which had a clearance less than 60 mL / min, intra-erythrocyte concentrations of metformin are higher for a creatinine clearance between 15 and 80 ml / min compared to that greater than 80 ml / min but are normal and with a significant difference between two groups of clearance 15- 30 ml / min and 30- 60 ml / min despite a reduced dose with p = 0.02.

Discussion : The intra-erythrocyte concentrations of metformin are higher for a creatinine clearance between 15 and 80 ml / min compared to that greater than 80 ml / min but are normal and with a significant difference between two groups of clearance 15- 30 ml / min and 30- 60 ml / min despite a reduced dose with p = 0.02. This is consistent with the results of Lalau JD et al., with the difference that they do not have found no significativity. A single case of lactic acidosis (LA) was observed in our study with an intra-erythrocyte concentration of high metformin to 4.56 µg / ml, a clearance 40 mL / min. In renal failure, its accumulation is proportional to creatinine ratio. Metformin is excreted by the kidney with a clearance 440-450 mL / min (normally 4 to 5 times that of creatinine).

Conclusion : In practice, metformin still occupies an important place in the treatment of type 2 diabetic patients with or without renal impairment. More fear than harm, clearance < 60 ml / min should not be a cons-indication

for the use of metformin as its intra-erythrocyte concentration don't rises considerably that if the clearance is < 30 ml / min and remains normal < 1.65 µg / ml.

I.   INTRODUCTION- CONTEXT JUSTIFICATORY

Type 2 diabetes and chronic renal failure are insidious diseases that evolve to low noise. Worldwide, diabetes is the leading cause of chronic kidney disease 44% in the US, 25% in Australia and 40% in France [1,2]. In Morocco, these two diseases are on the rise and represent a real public health problem. The prevalence of diabetes in Morocco reached 9% of the adult population over 20 years of which 50% remain undiagnosed [3]. Several factors appear to explain this situation : poverty and its corollaries (sub-medicalization, self-medication, illiteracy), sedentary lifestyle, poor eating habits sometimes influenced by the socio-cultural realities, consanguinity, the difficulties of proper diabetes care including therapeutic education of patients. Diabetes is the leading cause of chronic kidney disease in Morocco or 32.8% depending on the outcome of the investigation on the prevalence and risk factors for chronic kidney disease (MaReMar) the wider investigation on the African continent and the Arab world, made in Morocco [4]. The same survey found that 2.9% of Moroccans are affected by chronic kidney disease.

The pathophysiology of type 2 diabetes appears to be dominated by insulin resistance and a lack of insulin secretion. Its management is based on the management of these two factors diabetogenic by physical activity, the dietary measures, the insulin sensitizers, and secretory insulin.

The insulin sensitizer medical treatment has always been based on two families of drugs that are biguanides and glitazones that are not used in many countries including Morocco. Metformin, biguanide discovery in 1957 is the oral antidiabetic medication, insulin sensitizer most prescribed in the world including Morocco, where it is available free to all the diabetic population in the health centers.

Excellent insulin sensitizer, metformin is a well tolerated drug [5]. However, its use requires a respect for its different cons-indications including renal function or ignorance of the state of renal function or with a creatinine clearance below 60 ml / min [6]. Metformin is excreted by the kidney with a clearance 440-450 ml / min (normally 4 to 5 times that of creatinine). The main risk in renal insufficiency is lactic acidosis (LA) by accumulation or Intoxication with metformin. In renal failure, its accumulation is proportional at rate of creatinine [7,8]. The determination of concentration intra-erythrocyte of metformin is sometimes essential. The intra-erythrocyte concentration of metformin is the best indicator for assessing the safety of this medicine on the one hand in terms of secondary effects frequency, and secondly in terms of the risk of complications (renal or hepatic) and overdose. Its half-life varies between 9 and 17 hours.

However, the use of metformin may be as beneficial in patients type 2 diabetes mellitus insulin-resistant with renal insufficiency because it improves their glycated hemoglobin (HbA1c) decreasing so the risk of cardiovascular events, but at what dose? Very few studies have been done on metformin and kidney failure, and many questions remain about the risk / benefit ratio and hence the interest of our study in Morocco in this Mediterranean population to determine and study the difference in concentrations intra- erythrocyte metformin in patients type 2 diabetes mellitus with or without renal impairment, and to determine the prevalence of lactic acidosis.

Objectifs
-   Determine and study the difference in concentrations intra- erythrocyte metformin in patients type 2 diabetes mellitus with or without renal impairment.
-   Determine the prevalence of lactic acidosis.

Patients and Methods

This is a descriptive and comparative study over a period of two years between January 2013 and December 2014, of 64 type 2 diabetic patients with their consent, divided into two groups, treated with Metformin alone or in combination with other oral antidiabetic (OAD) and or insulin. All patients were seen for the first time in the department of Endocrinology, Diabetology and Metabolic Diseases, of University Hospital Hassan II of Fez in Morocco for several reasons (imbalance of diabetes, therapeutic education, surgeries).

The first group comprised 32 patients with impaired renal function, including all stages of renal insufficiency with creatinine clearance lower limit or equal (≤) to 80 ml / min. The second group was also composed of 32 patients without renal failure with a strictly higher creatinine clearance 80 ml / min. The creatinine clearance was estimated using the simplified MDRD formula (Modification of the Diet in Renal Disease).

The exploitation of medical records allowed collect data on patient history, clinical examination, the results of biological tests including creatinine clearance, intra-erythrocyte concentration of metformin, pH and lactate blood. An intra-erythrocyte concentration Metformin high (> 1.65 µg / ml), low blood pH (< 7) and high lactate (> 5 mmol / l) were considered pathological.

The determination of concentrations plasma and intra-erythrocyte of metformin was systematic in all patients fasting before taking metformin of morning. This concentration is known residual concentration.

The blood pH and lactate were not systematically made except before the association digestive clinical signs of lactic acidosis (AL): diarrhea, vomiting, abdominal pain. This is because of the precariousness of reagents available in the laboratory and the low socioeconomic status of our patients.

Statistical analysis was performed by Epi Info Version 7. The significance threshold is considered positive if p is less than 0.05.

II.   RESULTS

Among 64 identified patients including 28 men et 36 women, to be a sex ratio of 0.78 in favor of women.

The average age of our patients was 53 ± 15 years, ranging between 18 and 75 years.

Renal failure was present in half of the cases or 50% of which 3.1% between 15- 30 ml / min, 78.1% between 30- 60 ml / min, and 18.8% between 60- 80 ml / min. None of the patients had terminal kidney failure.

Lactic acidosis was present in 0.01% of cases.

The time evolution of the diabetes and the duration of treatment with metformin were ≤ to 5 years in 57.8% of cases.

Abdominal or central obesity was present in 73.44% of cases.

The intra-erythrocyte concentration metformin was proportional to the age of our patients without exceeding the upper limit of normal (*Figure 1=Fig1*).

The intra-erythrocyte concentration metformin was proportional to the stages of renal insufficiency our patients without exceeding the upper limit of normal (*Figure 2=Fig2*).

The intra-erythrocyte concentration of higher Metformin was found with the lower dose of metformin (1 g / day) and creatinine clearance 15-30 ml / min (*Figure 3=Fig »*).

The intra-erythrocyte concentration metformin was inversely proportional to HbA1c which was higher (9% at least) and in of creatinine clearances > 30 ml / min (*Figure 4=Fig4*).

III.   DISCUSSION

Very few studies have been done on metformin and renal failure including plasma concentrations and intra-erythrocyte in renally impaired patients with diabetes to assess the risk / benefit of treatment with metformin. In our study, the intra-erythrocyte concentrations of metformin are higher for a creatinine clearance between 15 and 80 ml / min compared with a creatinine clearance greater than 80 mL / min but they are normal and with a significant difference between two creatinine clearance groups of 15- 30 ml / min and 30- 60 ml / min despite a reduced dose of metformin with $p = 0.02$ *(Fig2)*. This is consistent with the results of Lalau JD et al., but they have not found of significant difference [9]. In renal failure, its accumulation is proportional to the rate of creatinine [7,8]. Metformin is excreted by the kidney with a clearance between 440-450 mL / min (normally 4 to 5 times that of creatinine).

The imbalance of diabetes was present in both groups of patients with a mean HbA1c 9.09 ± 1.9 in the group with renal failure against 9.17 ± 1.7 in the group without renal insufficiency *(Fig4)*. No significant difference was found between the two mean HbA1c with $p = 0.26$. This imbalance of diabetes in our study confirms the data from the study conducted in the population Maghreb (Morocco, Algeria, Tunisia) where diabetic patients were not well balanced with a HbA1c > 9% [10]. Moreover, we note a better balance of diabetes (HbA1c 6.2%) and normal levels of triglyceride to a creatinine clearance between 15 and 30 ml / min where the intra-erythrocyte concentration is highest but normal *(Fig4)*. This normal HbA1c could be explained by the underestimation of HbA1c at this stage of renal failure.

The biguanides have been introduced in the treatment of diabetes in 1950 [11]. The drugs are derivatives of the alkaloid a herb used in traditional medicine for centuries: Galega officinalis, also called false indigo or rue de goat, or French lilac. The effect galactagogue and effect diuretic of the dry plant were known since the middle ages [12]. The highlighting antidiabetic properties of its main alkaloid galegine, led to the synthesis of three compounds: phenformin, buformin and metformin. The metformin was developed in 1957 and today represents one of the most prescribed oral anti-diabetic in the world, with more than 25 million patients in the United States in 2000. The mode of action of metformin is not fully understood, but it was speculated that it could increase the action of insulin to, or promote the binding of the hormone on peripheral receptor sites [13]. This increase in insulin sensitivity appears to result from an increase in the number of insulin receptors on the cell surface [14].

The metformin is a small molecule (molecular weight 165 Da), very hydrosoluble. After an incomplete intestinal absorption (estimated at 60%), the peak plasma concentration is reached in six hours. The molecule is distributed according to a two-compartment model in the plasma sector and in the intracellular space, including intra-erythrocyte. Its protein binding is low, less than 20%. It is absorbed relatively slowly, over a period of up to more than 6 hours. It is excreted in the urine with high renal clearance is approximately 450 ml / min. The first stage of elimination of metformin is short, the half-life of the drug ranging between 1.7 hour and 5 hours. The terminal elimination phase is long, where are eliminated 4% to 5% of the absorbed dose, the half-life ranged then from 9 and 17 hours [11].

La metformin has shown efficacy in reducing mortality and reduction of cardiovascular complications in patients with diabetes type 2 with overweight [15]. Its effects are manifold: it promotes the hypoglycemic action of insulin by reducing insulin resistance; it increases the utilization and storage of glucose in muscle as glycogen; decreases intestinal absorption of glucose, the intestinal production of glucose and hepatic glycogenolysis [16,17]. These actions are related to complex cellular effects and poorly understood: the molecule is in the mitochondria where it induces an incomplete inhibition of the complex 1 of the respiratory chain and thereby decreases the oxygen consumption of hepatocytes; it activates AMP kinase (AMPK). The Phosphorylation of AMPK suppresses the production of glucose glucagon dependent and increases glucose uptake in muscle and hepatocytes [17]. It induces an increase of oxidative phosphorylation and beta-oxidation of fatty acids. Furthermore, the metformin modifies the opening of the mitochondrial transition pore and this action prevents apoptosis induced by hyperglycemia [17,18]. The cellular uptake of metformin is ensured in hepatic cell by a cationic transporter, organic transporter 1 (OCT1) and in kidney cells by OCT2. The tissue distribution of OCT1 is almost identical to that of metformin. The affinity of OCT1 for phenformin and buformin is higher than for metformin and this difference in the intracellular transport capacity could explain the lower toxicity [19]. Genetic polymorphisms of OCT1 modify the hypoglycemic effects of metformin and its pharmacokinetic characteristics [20,21].

The metformin is a drug well tolerated [5] and this is explained by the low affinity of transporter OCT1 with metformin [19]. In the absence of overdose or intoxication, side effects of metformin are mostly minors, of disorders digestive very common (> 1/10) especially at the beginning of treatment: dyspepsia, nausea, vomiting, diarrhea, bloating, flatulence and anorexia. The risk of accumulation of metformin and lactic acidosis (LA) increases with the degree of renal function impairment. This is a rare but serious complication resulting in death in about 50% of cases [12].

The lactic acidosis is characterized by elevated blood lactate concentration (> 5 mmol / l), decreased blood pH, electrolyte disturbances with an increased anion gap and elevation of report lactate / pyruvate. When the use of metformin is implicated, the plasma concentration of the drug generally proves greater than 5 µg / ml. The lactic acidosis is much rarer with the metformin than the phenformin, with a prevalence of 10 to 20 times lower [22,23]. The phenformin and buformin were removed from the market due to the occurrence of fatal lactic acidosis whose frequency was encrypted between 40 and 65 cases per 100 000 patients per year. In patients receiving metformin, the reported incidence of lactic acidosis is very low (approximately 0.03 cases per 1000 patient-years, with approximately 0.015 fatal per 1000 patient-years) and occurs primarily in diabetic patients with significant renal insufficiency, including renal disease and often organic renal hypoperfusion associated with multiple medical and surgical problems and multiple concomitant use of drugs [24]. A single case of lactic acidosis with digestive signs was observed in our study in an elderly woman, diabetic, hypertensive, on an intra-erythrocyte concentration of metformin high almost three times normal with a creatinine clearance 43 ml / min. Several cases of lactic acidosis induced by metformin have been reported without kidney disease [25].

The mechanism of occurrence of lactic acidosis in patients receiving metformin is complex and multifactorial. On the one hand, the metformin induces a decrease in intestinal absorption of glucose, which is then metabolized into lactate splanchnic level. On the other hand, inhibition of hepatic gluconeogenesis prevents the metabolism of lactate produced by the muscle [26,27]. Finally and above the inhibition of complex 1 of the respiratory chain accelerates glycolysis, directs the metabolism towards the anaerobic function and results in a decrease in oxygen consumption (VO2) and lactate production [18]. The effect of metformin on VO2 was recently confirmed in a clinical study in 24 patients with severe lactic acidosis. The VO2 calculated from the arteriovenous difference measurements and cardiac index was very significantly reduced at admission and was inversely correlated with blood lactate [28].

Cases of metformin overdose of were reported, some involving ingestion of more than 50 g [11]. About 32% of metformin overdose were accompanied by lactic acidosis. The metformin is suitable for dialysis, its speed of elimination can reach 170 ml / min under good hemodynamic conditions. Metformin having a low molecular weight and low protein binding is a toxic readily dialyzable [7.29]. The hemodialysis may therefore be helpful to remove the accumulated metformin in suspected drug overdose. Consequently, patients with serum creatinine levels above the upper limit of normal according to the age should not receive metformin [6]. In elderly patients, It is necessary to carefully adjust the dose of metformin to determine the minimum dose which allows to obtain a balance glycemic, because aging is associated with reduced renal function.

IV. CONCLUSION

In practice, metformin still occupies an important place in the treatment of type 2 diabetic patients with or without renal impairment because it allows the reduction of cardiovascular complications in patients with diabetes type 2 with overweight, metabolic syndrome and the reduction of mortality. More fear than harm, clearance < 60 ml / min should not be a cons-indication for the use of metformin as its intra-erythrocyte concentration don't rises considerably that if the clearance is < 30 ml / min and remains normal < 1.65 μg / ml. The lactic acidosis although rare, is a more serious complication and fatal of its accumulation. However, in elderly patients and patients with renal insufficiency, the gradual adaptation of metformin doses with monitoring of digestive signs, of renal function and of concentration intra-erythrocyte of metformin seem unavoidable.

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Annexes : Figures

The intra-erythrocyte concentration metformin was proportional to the age of our patients without exceeding the upper limit of normal.
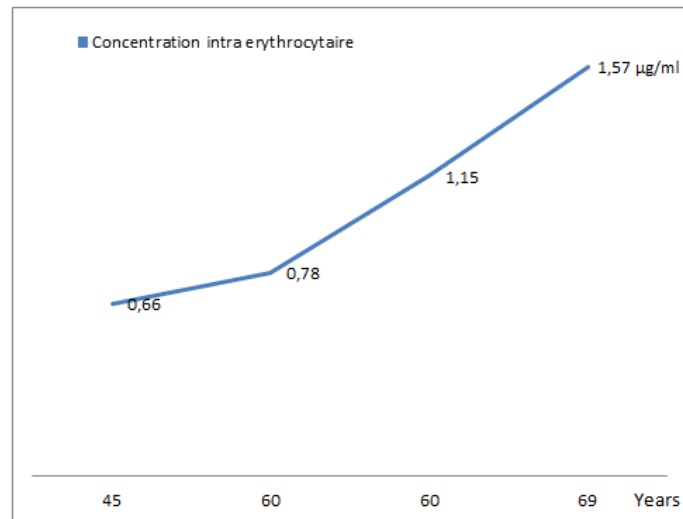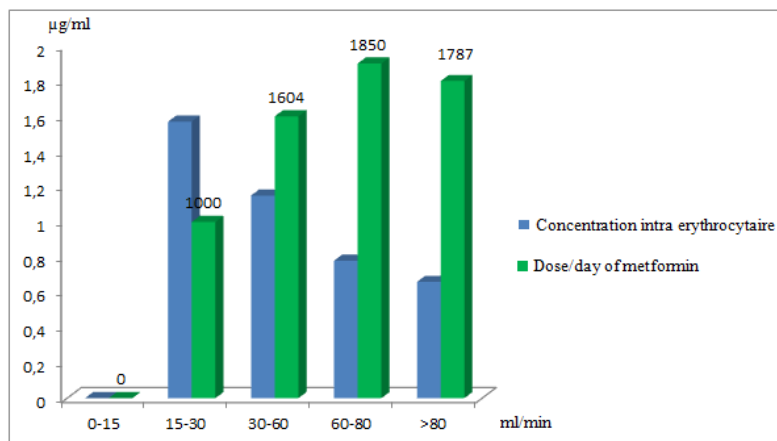
Figure 1 **:** Intra-erythrocyte concentration of metformin according to the age of patients

The intra-erythrocyte concentration metformin was proportional to the stages of renal insufficiency our patients without exceeding the upper limit of normal.
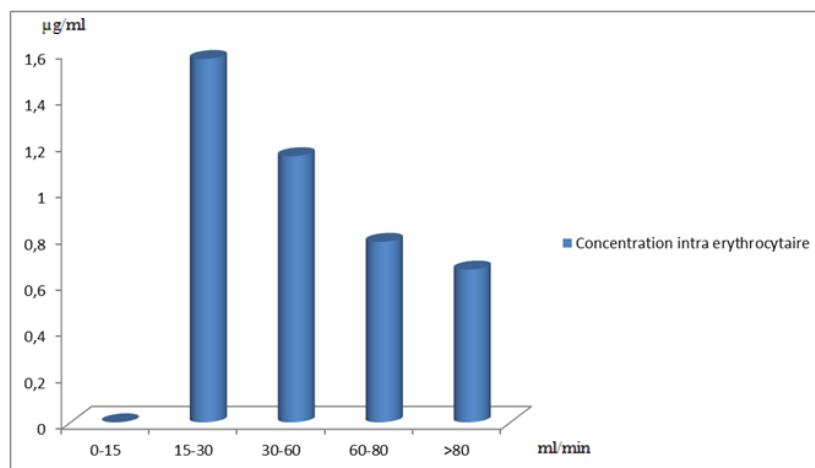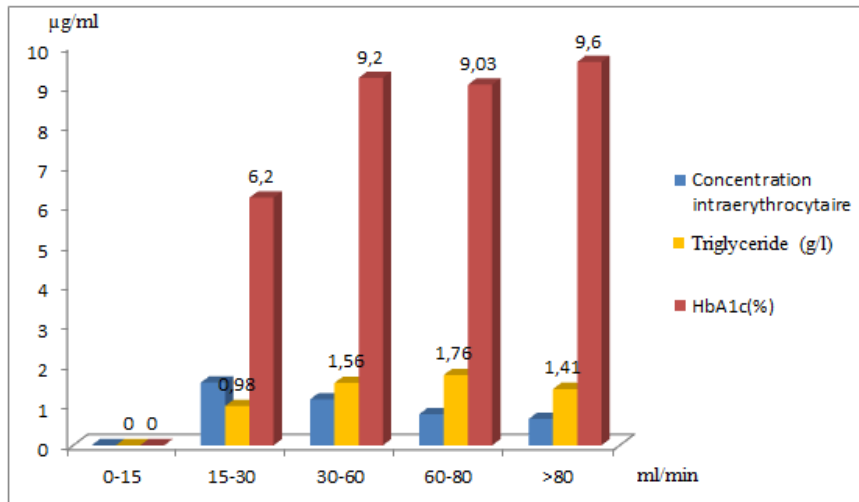

Figure 2 **:** Intra-erythrocyte concentration of metformin according to the creatinine clearance

The intra-erythrocyte concentration of higher Metformin was found with the lower dose of metformin (1 g / day) and creatinine clearance 15-30 ml / min.

Figure 3 **:** Intra-erythrocyte concentration of metformin according to the creatinine clearance and to the dose of metformin

The intra-erythrocyte concentration metformin was inversely proportional to HbA1c which was higher (9% at least) and in of creatinine clearances > 30 ml / min. A better balance of diabetes (HbA1c 6.2%) and normal levels of triglyceride to a creatinine clearance between 15 and 30 ml / min where the intra-erythrocyte concentration is highest but normal.

Figure 4 **:** Intra-erythrocyte concentration of metformin according to the creatinine clearance, to the HbA1c and to the dose of the triglyceride