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# The Effect of Sex and Gender on Diabetic Complications.

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**Running title**: Sex-gender and diabetic complications.

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**Abstract:**

**Background and Aims**: While in non-diabetic people the risk for cardiovascular disease is higher in men, diabetes completely reverts this gender difference conferring to women a greater burden of cardiovascular complications. Additionally, all risk factors associated with cardiovascular diseases appear to be more active in diabetic females than in their male counterparts. The reasons of this different impact of diabetes between genders have not been completely clarified. Aim of this review is trying to clarify these issues in a sex and gender perspective.

**Results:** Possibly women arrive later and in worse conditions to the diagnosis of diabetes, receive both diagnostic and therapeutic supports in a lesser measure and, finally, reach therapeutic goals as recommended by guidelines in a lesser extent. Further aspects of sex-gender differences in diabetic complications are represented by a more frequent prevalence of drug side effects in women, as well as by increased resistance to the action of drugs used in prevention or in the therapy of cardiovascular diseases. As to microvascular complications, the issue of sex-gender differences is even more complex, with some important differences emerging in experimental models *'in vitro'*, as well as in human pathology *'in vivo'*. The main problem, however, also in this case, is that it is difficult to differentiate how common pathogenetic mechanisms acting in diabetes may differently impact between genders.

**Conclusions:** In conclusion what is clearly evident is that diabetes represents a *'risk magnifier'* for the damage of both micro and macrovessels differently in men and in women. This issue deserves, therefore, a more careful approach from people involved in both clinical aspects and research regarding diabetes and its complications, in a sex-gender oriented perspective.

**Keywords:** Diabetes, sex-gender differences, adverse drug reactions, diabetic macroangiopathy, diabetic neuropathy, diabetic nephropathy, diabetic retinopathy.

## INTRODUCTION

Before entering into the core of this review a more precise notification is needed: it is rather difficult to clearly divide sex from gender. Due to the numerous interactions existing between sex (biological aspect of femininity and masculinity) and gender (social construct that generally transforms a female into a woman and a male into a man, giving different access to education and occupation), it seems appropriate a term which encompasses all two definitions. In this review we will, therefore, adopt the sex-gender terminology, instead of using sex or gender in an interchangeable manner [1]. As widely known diabetes is a known factor associated to an excess risk of cardiovascular mortality and, in this context, there are evidences highlighting that diabetic women are at a higher risk, especially in postmenopausal period [2]. Mortality and disability after a first vascular event is higher in women and there are evidences reporting that women receive less medical care regarding cardiovascular complications even in presence of diabetes. As the readers will realize, when gradually they'll step further into this review, the reasons of the increased burden of cardiovascular complications in diabetic women are to date not completely understood and many factors have been advocated trying to explain such gender effect. We'll bring evidences, from

published studies, that: first, women come later and in worse clinical conditions to diagnosis of diabetes. Second, they are more obese at diagnosis and reach in a much lesser extent guideline target goals as to metabolic, lipidemic or blood pressure control, [3] third, they have a lesser chance of receiving all the diagnostic and therapeutic measures than their diabetic male counterpart, even if it is well known that mortality after a first cardiovascular event is more elevated in diabetic women [4,5]. Finally, anti-aggregating and hypotensive drugs seem to be less efficacious in diabetic women, while side effects of hypoglycemic agents seem to be more frequent in females [6], (see further in the review).

Regarding diabetic microvascular complications, studies focused on sex-gender differences are indeed scarcely represented either at preclinical or clinical level, mainly due to the well known limitations of patient inclusion criteria in trials, but also due to the difficulty of dissecting genetic and environment interactions. In addition drug treatment outcomes in micro-macrovascular complications need large scale trials to evaluate the differences in treatments in function of sex-gender, not only in the light of the outcome, but also of sex-gender oriented side effects of these drugs. Certainly, the lack of our capacity to target directly the mechanism initiating the disease, instead of the epiphenomenon, is the cause of the partial failure in the control of diabetic microvascular complication and this is true in sex-gender oriented medicine as well.

As a preliminary for this review we first propose a schematic table which represents all main aspects that will be further discussed in the text, regarding macro and microvascular complication in diabetes mellitus in a perspective of sex and gender (Table 1). This table shows what possibly are the main differences linked to sex and respectively to gender, valid for any medical or clinical condition. Finally, when concluding this review, we'll try to fill this table's preliminary scheme with proper contents, in relation to the specific clinical situations represented by diabetes and by its complications.

| **Sex** | **Gender** |
|---|---|
| • Anatomical differences | • Social position |
| • Hormonal milieu | • Occupation |
| • Pregnancy | • Education |
| • Weight/body composition | • Physical activity |
| • Differences in renal function | • Habits (smoking, alcohol intake,...) |
| • Differences in drug action (kinetics, dynamics, side effects) | • Access to health service opportunities |
| • Differences in life expectancy and in aging | • Different representation in RCTs recruitment |
| • Blood biochemistry | • Influence by physician's gender in medical procedures |
| • Genetic predisposition/Heredity to CVD/neoplasms | • Difference in disease symptoms/signs awareness |

**Table 1 –** Sex and gender differences related to the clinical issues represented by any medical condition.

## SEX-GENDER DIFFERENCES FOR RISK OF CARDIOVASCULAR DISESES (CVD) IN DIABETES.

A dated but fundamental observation regarding the Framingham cohort points to a markedly higher risk of cardiovascular diseases in diabetic women than in men, since diabetes, when compared with no diabetes, raises the risk of ischemic heart disease by about two times in males and by about four times in females [7] and this observation was further evidenced by more recent studies [8-11]. In summary a large amount of evidences are now in agreement with a first conclusive remark according to which diabetes raises the risk for atherosclerotic complications by a significantly greater factor in women than in men. Why does this happen and, in particular, what's there at the basis of this sex-gender difference, even if not completely understood, is becoming more and more clear.

A first important issue is that the amount of factors which altogether build up the attributable risk for diabetes comes from a quite different background in men and in women. In these latter, indeed, gestational diabetes, polycystic ovary syndrome, history of preeclampsia, premature menopause, may cluster to give a substantial amount of attributable risk of diabetes [12-14]. In men the background condition is obviously different and the contribution of diabetes to global attributable risk interplays with more classic risk factors such as dyslipidemia, smoking, hypertension, central obesity, physical inactivity etc. as individuated, for instance, by the INTERHEART study, even if all

these classical factors are, obviously, observed also among women [15].

To better individualize the difference in attributable risk of diabetes and of its vascular complications, an important role is played by sexual hormones which modulate the risk of precocious atherosclerosis since, as known, estrogenic hormones contribute in protecting endothelia against atherosclerotic damage. Diabetes appears to obviate these protective effects exerted by female sex hormones [16-18], and, consequently, anticipates the excess risk of atherosclerotic events in postmenopausal women who abruptly lose their hormonal protection. As a consequence of this, the absolute cardiovascular event rates at younger age are higher in men with or without diabetes [19,20]. In other terms diabetes acts as a risk 'magnifier' in women in a strong dependence to specific life's time periods, opening some 'high-risk windows', especially during the perimenopausal period, when, as widely known, the risk for cardiovascular events is higher even in non diabetic women than in men [21]. This amplifying effect of diabetes has been particularly observed regarding the risk of ischemic heart disease [22], of post-ischemic heart failure [10], as well as of early mortality after myocardial infarction [23]. A first conclusion is therefore that difference in diabetes related excess risk of cardiovascular diseases is linked to some life periods [24]. In this context a further example is brought about by gender difference in mortality risk after stroke. As known and widely demonstrated by the majority of epidemiological studies, both the risk and the incidence, of this vascular event is greater in men than in women in general population [25], while, on the contrary, the mortality or disability rates after stroke seem to be heavier among the women [26,27]. In this case, however, after taking into account the confounding effect of age, many differences disappear, underscoring the fact that the higher burden of disability and of cardiovascular events in women is, at least partly, due to their higher life expectancy. After these general considerations, however, it is necessary to review the impact of any single risk factors on the construct, building up the overall background risk excess associated to diabetes. Beginning from considering the risk factor represented by elevation in glucose levels, on a population perspective, there are several lines of evidence suggesting that females have higher prevalence of impaired glucose tolerance, while males present a higher prevalence of impaired fasting glycemia [28-33]. A possible reason of this could be found in the fact that diagnosis of diabetes is done later in women and a more consistent prevalence of obesity is being observed at diagnosis [34]. On the other hand, however, the rate of women is much less represented in trials testing the relation between disglycemic states and cardiovascular events [14]. This represents an evident contradiction since while impaired glucose tolerance is more evident among women, trials concerning intervention in CVD of people with altered glucose metabolism including diabetes would be thus expected to recruit more women than men. On the contrary, the both the number and the access to women to intervention trials appear underestimated, so representing an overt limitation in any conclusion about the relationship between glucose levels and clinical cardiovascular outcomes in women [35]. All this is even more cogent from the very recently published EMPA-REG trial which has demonstrated the relevant protective effect of the SGLT-2 antagonist emapagliflozin against the risk of cardiovascular deaths in a cohort high-risk diabetic patients, composed of about the 75% by male subjects [36].

**Classical risk factors**

A further aspect useful to explain why diabetic women are more at risk than men is that classical risk factors for atherosclerosis appear to be are more active in diabetic women than in diabetic men. In this context it has been observed that abdominal obesity is more prevalent in women with diabetes [37,38]. The same trend was observed as to hypertension whose prevalence is greater in women than in men, at least in the elderly, [39], and, again, both the incidence and prevalence of hypertension are higher in diabetic women compared to diabetic men [7,37,38]. In addition, it is noteworthy that high blood pressure levels exert a greater effect on left ventricular mass of women [40] and this observation must be linked with a further demonstration that women, either diabetic and non-diabetic, seem to be more vulnerable than men to the risk of hypertension [41].

With regard to a further classic risk factor for atherosclerosis i.e. dyslipidemia, some data seem to support the hypothesis that diabetic women present a worse lipidemic profile than diabetic men as represented by higher levels of triglycerides and lower levels of HDL-cholesterol [3,42]. Finally, some epidemiological studies indicate a greater impact of diabetic dyslipidemia on cardiovascular risk in diabetic women than in diabetic men [9, 38].

Further risk factors which are more linked to female sex in diabetes are oxidative stress and hypercoagulability. These latter are, as known, more represented in patients with diabetes, and again diabetic women seem to be more vulnerable to these risk factors [43].

**Other clinical aspects**

When dealing with sex differences in the presentation of cardiovascular diseases in diabetes, two further aspects have to be taken into account: the first is a sexual dimorphism in the presentation of symptoms of cardiovascular events. As known diabetic women present atypical or attenuated symptoms of both myocardial infarction [44] and stroke [45] and all this translates into a later and more inefficacious diagnostic and therapeutic approach in female diabetic patents. A second point to be taken into account is the different approach regarding fundamental therapeutic strategies. Diabetic women are less frequently treated with coronary revascularization strategies when affected by acute myocardial infarction [46] and with tissue plasminogen activator therapy after acute ischemic stroke [47].

Finally a particular consideration must be given to the role played by different gender associated genetic profiles apt to promote differences in the incidence of macrovascular complications in diabetes. As to this aspect, an interesting paper of Silander *et al.* [48] concerning the FINRISK cohort has recently highlighted the issue of gender differences in genetic risk profiles for cardiovascular disease. The conclusion is that genetic risk loci for CVD are more readily detectable in women, while they are more confounded by environmental/lifestyle risk-related factors among men. Since diabetes undoubtedly represents an additional 'confounding variable' when assessing the global risk for cardiovascular diseases, it is obvious that investigating the genetic effects on gender differences in risk of cardiovascular diseases may become more difficult in diabetic patients.

A further important question is whether diabetes is associated with a different excess risk of mortality after cardiovascular events between genders. Even if the finding is not definitively proven, some reports suggest a greater case fatality rate after ischemic stroke in women [49] while according to a German study by Icks *et al.* men seem to be paradoxically protected by diabetes [50]. Interestingly this finding has been also recently confirmed by data collected in Tuscany, Italy [51] and may be explained with the fact that, while diabetic patients are more closely monitored because of this condition, females seem to experience CVD episodes in worse medical conditions than men. Furthermore there is to note that both in USA and in Europe there has been a continuous decline in deaths after coronary heart diseases in the total population mainly due to better life conditions. In the light of this observation, a very recent paper concerning the Danish cohort of diabetic patients from the Steno Memorial Hospital of Copenhagen followed up since 2002 to 2010, showed that mortality for CVD is more rapidly decreasing in diabetic population, probably due to a more incident medicalization of diabetic individuals, and that this trend is more evident in men, in keeping with what suggested by similar evidences [52].

**Disparities between genders in diagnosis and treatment of diabetic patients**

A further aspect which suggests important differences in the risk of CVD between genders in diabetes is originated by the huge amount of data from literature indicating that a disparity of both prevention measures and treatments is present between genders since diabetic women, other than arriving later to the diagnosis of diabetes, are treated worse than diabetic men, reach, in a significant lower rate, standard target treatment goals, as to glycemic or lipidemic control targets, and receive less frequently than man a prescription of antiplatelet therapy [53-59]. In this context, a recent Italian study, concerning a wide national dataset regarding hospital diabetes outpatient clinics has demonstrated that quality of care process indicators were significantly different between genders and in particular women were more likely than men to have HbA1c>9.0% in spite of insulin treatment, as well as to have LDL cholesterol (LDL-C) ≥130 mg/dL in spite of lipid-lowering treatment or, finally to have a body mass index (BMI) ≥30 kg/m$^2$. Finally, women were less likely to be monitored for foot and eye complications [3]. This issue is even more complicated by the recent indication that there are gender differences in the relationship between diabetes process of care indicators and cardiovascular outcomes [60]. Adherence to Guideline Composite Indicator, a process indicator including one annual assessment of HbA1c and at least two among eye examination, serum lipids measurement and microalbuminuria, was indeed found to be a significant predictor of lower risk of cardiovascular events (especially hospitalization for myocardial infarction), with a greater prediction capacity more often in men than in women [60]. These findings contribute to raise awareness among healthcare professionals and policy makers towards gender-sex aspects in the definition of guidelines indicators for diabetes, when tested in population studies.

All this is even more complicated by the suggestion that some treatments are differently efficacious between genders, independently from the presence of diabetes. A recent paper, for instance, indicates that target 1-year-glycemic response (HbA1c≤7%) is achieved in a significantly higher proportion of males than females after therapy with GLP-1 agonist exenatide [61].

Side effects of hypoglycemic drugs, on the contrary, even if equally represented, are substantially different between genders [54]. Further gender differences concerning antidiabetic treatment have

been extensively reported. Fertile women in the second half of their menstrual cycle require a higher insulin dosage to maintain metabolic control [62]. Furthermore, hypoglycemic events seem to be less frequent in women than in men at least in type 2 diabetes [63] Additionally, insulin therapy can increase bone fracture risk probably due to the secondary effect of hypoglycemia which increases the risk of falls especially in the elderly [64] and its impact could be more relevant in women because osteoporosis has a higher prevalence in women than in men. Some patients with type 2 diabetes are reluctant to start insulin and may delay it. This process, called "psychological insulin resistance", affects at a greater extent females (32.0%) than males (21.1%) [65] As known metformin is involved in lactate generation, which leads to the elevation in levels of blood lactate [66], especially in patients with impaired renal function [67]. Interestingly, plasma lactate levels have been found significantly higher in female than in male patients treated with metformin, with the highest level of lactate in premenopausal women with SLC22A2 gene TT variant [68], and thus, women with diabetes should deserve a greater caution than men when treated with metformin, with the aim of preventing lactic acidosis. Moreover, as to metformin, interestingly a recently published paper has shown that female patients with complications seem to be more responsive than males to the CV protection offered by metformin [69].

Thiazolidinediones therapy raises the risk of bone fractures more frequently in women [70], while dipeptidyl-dipeptidase-4 (DPP4) inhibitors have, on the contrary, been reported to be associated with a reduced risk of bone fractures, even if independently by sex-gender [71].

Moreover, a more intriguing aspect is the possible effect of antidiabetic therapy and cancer. In this case metformin seems to have a protective effect from breast cancer in women [72], while the long acting insulin analog glargine seems to be related with an increased risk of breast cancer [73] even if this latter finding is extremely controversial.

An important class of antihypertensive and heart protective drugs such as those which target the RAAS [(angiotensin converting enzyme inhibitors (ACEs) and angiotensin receptor blockers (ARBs)] may prevent CV events more efficaciously in men than in women [74]. A similar difference has been observed as to another important drug class namely the anti-aggregating therapy such as aspirin. A meta-analysis has, indeed, demonstrated that women are less responsive to aspirin treatment than men in trials aimed at primary prevention of cardiovascular events [75,76].

## MICROVASCULAR COMPLICATIONS

While macrovascular complications are not peculiar of diabetes, the condition *sine qua non* to develop diabetic microvascular complications is diabetes-associated chronic hyperglycemia. Typically microvascular complications are diabetic nephropathy (leading cause of end stage renal failure), diabetic retinopathy (leading cause of blindness) and diabetic neuropathy (able to increase the cardiovascular disease and being the leading cause of non traumatic amputations at lower extremities).

### Diabetic Nephropathy

Diabetic nephropathy is a progressive disease caused by angiopathy of capillaries in the kidney glomeruli and/or tubule-interstitial and is a prime indication for dialysis in most Western countries. Nephropathy increases also the cardiovascular risk, as it is well known that estimated glomerular filtration rate (eGFR) below 60 ml/min is considered a cardiovascular risk factor besides being a sign of progression to end stage renal failure. In the past it was generally agreed that the disease was characterized by an evolution from micro to macro albuminuria and then to the decrease in GFR and progression to end stage renal disease. Nowadays both in type 1 and in type 2 diabetes it is clear that the decrease in eGFR and the progression to end stage renal disease may occur without microalbuminuria preceding eGFR decrease, thus indicating the need to calculate eGFR and it is therefore mandatory to pay attention to its modifications also in patients whose urinary albumin excretion is within the normal range [77]. Within the past 20 years despite implementation of treatments that were presumed to be protective for kidney function, diabetes mellitus continues to rank as the number one cause of ESRD [78]. Recently it has become clear that diabetic nephropathy is a heterogeneous entity, conditioned by several factors, including diabetes type, genetics, social status, blood glucose and pressure levels, environment, gender etc. As to gender it is to recall that sexual hormones might have a pivotal role in the pathogenesis of glomerulopathy and tubulointerstitial lesions in diabetes and sex-gender impact on diabetic nephropathy has been extensively reviewed previously [79-82].

Generally, if women seem to be more protected against the development and progression of renal diseases compared to men, however the advantage is less evident in diabetic nephropathy than in non-diabetic kidney diseases [82,83]. Studies in humans, animal models, and cell cultures have provided evidence that microvascular diabetic injury is exacerbated by poor glucose control, and this

applies also to renal damage. Renal diabetic injury may be indeed exacerbated by poor glycemic control when sex-gender differences are most evident. It has been established that under good metabolic control diabetic women are more likely to develop diabetic nephropathy than men, whereas the opposite happens under poor metabolic control [84]. Elevated blood pressure and particularly systolic [85] has a clear role in kidney function deterioration and women over 60 years have greater hypertension prevalence than men [86] while hypertensive women have a lower control of blood pressure than men especially if they are diabetic [87-88]. Several studies have moreover suggested that 17β-estradiol ($E_2$) protects women from diabetic kidney disease, at least in part by regulation of transforming growth factor beta (TGF-β1). TGF-β1 serum levels are higher in men than in women [89] and rodents animal models with low $E_2$ levels, either diabetic or not, show increased renal expression of TGF-β1 [90]. $E_2$ supplementation was reported to be renoprotective by attenuating glomerulosclerosis and tubulointerstitial fibrosis by reducing extracellular matrix (ECM) synthesis and increasing ECM degradation in streptozotocin-induced diabetic rats [91]. In db/db mice tamoxifen and $E_2$ treatment decrease TGF-β1-mRNA expression and increased mRNA expression of the estrogen receptor subtype beta protein in isolate podocytes [92]. Finally, in streptozotocin-induced diabetic Sprague-Dawley rats, $E_2$ was able to controls TGF-β1 signaling and expression [93]. Several are the mechanisms proposed to explain these effects of $E_2$ on TGF-β1 expression [94,95]. When type 1 diabetes is first diagnosed during adolescence, some differences were present between men and women as to the cumulative incidence of diabetic kidney disease, suggesting, again, a role for sex hormones in mediating this difference [96]. This difference seems, moreover, to be highly age dependent starting at puberty [97,83]. In conclusion the concept arising from these observations might be that, regarding the susceptibility to kidney disease, 17*β*-estradiol seems to have a positive role' and testosterone a negative one, but the matter is far more complex. In addition, oral contraceptives containing high doses of estrogens increase the risk of diabetic nephropathy, whereas lower estrogen doses have no influence on renal function [84].

As estrogens seem to have a protective action against the development and progression of cardiovascular diseases [97-100], considering also the supposed protective effects exerted by $E_2$ on the diabetic kidney by interfering with TGF-*β1* signaling, an obvious conclusion would be that $E_2$ administration to diabetic patients might be able to prevent or at least ameliorate diabetic kidney damage. Unfortunately no large RCTs have done on this subject and, in any case, there are several theoretical limitations in the use of $E_2$ replacement therapy to prevent diabetic kidney disease. First, diabetic men have already higher levels of $E_2$ than the non diabetic subjects [101]. Indeed, experimentally, male diabetic rats show over-expression of kidney aromatase inducing a higher rate of intrarenal conversion of testosterone to $E_2$ [102]. Second, kidney is able to synthesize steroid hormones and local kidney variations in $E_2$ and testosterone concentrations could affect the above mentioned expression and signaling of TGF-β1 independently from serum hormone concentration.

However, independently from a different hormonal interaction, data on the sex-gender issue in diabetic patients are not univocal because some studies suggest that male gender remains a risk factor for the development of micro- and macroalbuminuria as well as for the progression of an established diabetic nephropathy. Racial and genetic factors do things even more complicated. In particular, Native Americans, Hispanics (especially Mexican-Americans), and African-Americans have a much higher risk of developing end-stage-renal-disease than non-Hispanic whites with type 2 diabetes. Also, genetic polymorphism could have a role to build up a sexual dimorphism in diabetic renal disease. Sex-determining region Y-box 2 (SOX2) is a transcription factor that plays an important role in the induction of pluripotent stem cells from somatic cells and the SOX2 gene is located in chromosome 3q26.33, in the linkage region of diabetes and diabetic nephropathy. A study carried out in 1120 patients with type 1 diabetes provided the evidence that in 1120 patients with type 1 diabetes mellitus (591 women, 529 men), single nucleotide polymorphism rs11915160 of SOX2 gene is significantly associated with DN (odds ratio [OR] = 0.720; P = 0.038) and end-stage renal disease (OR= 0.686; P = 0.034) in women but not in men suggesting that SOX2 genetic polymorphism has gender-specific effects on diabetic nephropathy [103].

Furthermore, the M235T polymorphism in the angiotensinogen gene increases the incidence of nephropathy only in type 2 diabetic male patients [104] and the angiotensin II type 2 receptor gene is involved in the development of kidney disease in type 1 diabetic men but not in type 1 diabetic women [105]. In addition, sexual hormones largely affect also RAAS system, a system that is deeply involved in the development and progression of diabetic nephropathy [106].

Interestingly, hyperglycemia induces an attenuation of effective renal plasma flow as well as an increase in renal vascular resistance and filtration fraction in normoalbuminuric, normotensive type 1 diabetic women but not in their male counterparts

[107]. This might, in part, explain the loss of female protection in presence of diabetes.

In younger women under 45 years of age, cigarette smoking seems to be an important risk factor for sudden cardiac death [108,109]. In any case there are conflicting results on sex-gender oriented effects of cigarette smoking on kidney function. This may reflect several factors such as, for instance, differences in smoking habits, different age distribution of males/females included into the studies, or the use of oral contraceptive and of hormone replacement therapy. Christiansen provided the first evidence that patients who have type 1 diabetes and who smoke have a higher risk to develop diabetic nephropathy [110], increasing the risk to develop microalbuminuria, and of accelerating the rate of progression from microalbuminuria to manifest proteinuria, [111]. Even passive smoking has been suggested to increase the chance of future type 2 diabetes in a population of young subjects and adult women [112]. Giving up smoking in patient with recent diagnosis of type 2 diabetes is associated with an amelioration of metabolic parameters, blood pressure and of renal albumin excretion within 1 year [113] showing that, in this context, the type of diabetes does not seem to play a role. Additionally, the negative impact of smoking on kidney function in patients with diabetes seems to be independent of the age of the patient as well as of the duration of the disease [114-117]. Recent findings [118] suggest that, similarly to what happens for coronary artery disease [119], some subjects are resistant to the adverse renal effects of smoking as a result of a yet largely unknown genetic background. This is confirmed by another recent study, the Bergamo Nephrologic Diabetes Complications Trial in which the DD-genotype of the ACE gene was strongly associated with microalbuminuria in smokers [120]. Some of us have recently identified that in young oral contraceptive-free women, smoking is able to increase all the oxidative stress factor commonly seen in males [121]. In that study we produced convincing evidence that regular cigarette smoking induces significant alterations in cardiovascular risk factors electively in young adult women and some of these risk factor might act also putatively at the kidney level.

Oxidative reactions are an essential part of the metabolic process because oxygen is the ultimate electron acceptor in the electron flow system that produces adenosine triphosphate (ATP). Problems may arise when electron flow and energy production become uncoupled, so oxygen free radicals (ROS) are excessively produced. When examining the key role of nitric oxide (NO) in cell signaling and its relation with ROS production, some studies have evaluated the sex–gender disparities in induced nitrosative stress. The better preservation of renal function during ischemia-reperfusion of the kidney is associated with high NO concentration and low peroxynitrite levels in females, whereas increased oxidative and nitrosative stress worsens renal damage in males.

Last but not least, in diabetes there is also a greater incidence of genito-urinary infections, caused by bacteria and fungi, particularly in females. Much of this morbidity, that might contribute if not directly at least indirectly, to kidney function's decline, remains often unrecognized, undiagnosed, and untreated in spite of the efforts done by diabetologists.

In summary, a conclusive answer as to whether sex-gender plays a role in the development and progression of renal disease in type 2 diabetes is still missing, and thus it urges to investigate the effect of sex-gender in a more detailed and precise manner also in consideration of the fact that diabetic nephropathy, even if associated with a small increase in urinary albumin excretion, increases the cardiovascular risk [77,122,123].

**Diabetic Retinopathy**

Diabetic retinopathy (DR) is one of the main causes of visual loss in subjects of age between 20 and 64 years [124]. Over the past few decades a number of clinical trials have confirmed that careful control of glycemia and of blood pressure can reduce the risk of developing diabetic retinopathy and delay its progression. In recent years, many new treatment options have been developed for clinical management of diabetic proliferative retinopathy and macular edema using laser-based therapies, vitrectomy and intravitreal corticosteroids or anti-vascular endothelial growth factors. In any case the results of these different treatments show that they are of limited benefits. New drugs and strategies are based on targeting a number of hyperglycemia-induced metabolic stress pathways, oxidative stress and inflammatory pathways, the renin-angiotensin system, and neurodegeneration, in addition to the use of stem cells and ribonucleic acid interference (RNAi) technologies. With such premises, although less than in other diabetic complications, also diabetic retinopathy shows sex-gender differences often not considered in the treatment.

The generally agreed risk factors for diabetic retinopathy are male sex, higher HBA1c level, longer duration of diabetes and higher systolic blood pressure [125]. In spite of the seemingly higher risk observed among males, different recent studies have questioned this sex-gender difference and controversial results are available in literature. In an old epidemiological review no significant correlations between retinopathy and gender have been reported [126], while a higher prevalence of retinopathy among women than among men has been described [127,128] even if with the finding that retinopathy was more

severe in men [129-131], although other studies suggests that, on the contrary, diabetic women have indeed more severe retinopathy with a higher probability than diabetic men to get major visual impairment [132]. Recently a clinic-based retrospective longitudinal study, including) Japanese patients with type 2 diabetes mellitus showed that females exhibit a significantly higher prevalence of proliferative diabetic retinopathy at baseline and that female gender is an independent risk factor for the development of diabetic retinopathy [133].

In addition, besides this gender controversy, it is generally agreed that diabetic retinopathy progresses during pregnancy [134,135], and that it is aggravated upon sex hormone administration, followed by a return to baseline conditions after the cessation of therapy [136], suggesting a certain influence of sex hormones in modulating the retinal damage in diabetes.

It is not excluded that further confounding variables may play a role in modulating sex-gender differences in the pathogenesis of diabetic retinopathy since some of us have shown that oral contraceptive may influence blood coagulation inducing several changes in hematological and plasmatic markers, modifying hormonal levels, endothelial function, inflammation indices and some redox state parameters [137] all of which might influence the progression of diabetic retinopathy synergistically with diabetes duration and poor blood glucose control.

As new therapies for diabetic retinopathy and its associated complications emerge, monitoring of new epidemiological data becomes more and more important to evaluate the impact and effectiveness of these therapies from a sex-gender perspective.

**Diabetic Neuropathy**

This is the most common diabetic complication since as much as 50% of both type 1 and type 2 diabetic patients have signs of diabetic neuropathy after 10-15 years of disease [138]. Diabetic neuropathy may be split into two clinical pictures: sensorimotor (SMN), or cardiovascular autonomic (CAN). While CAN is more associated with cardiovascular mortality, SMN may be the dreadful contributory cause of diabetic foot disease with resultant diabetic ulcerations and lower extremities' amputations.

Few small studies indicate that men with type 2 diabetes have more neuropathic complications than women [139-143]. In line with these results, lower limbs' amputation rate is more frequent in men compared to women [144] while, in spite of having a lower prevalence rate of this complication, women seem to have higher mortality associated with diabetes-related lower limbs' amputations. [145,146].

Certainly, there are genetic and population-associated differences, since in the Asiatic population the prevalence of diabetic neuropathy is higher in women than in men, while in Caucasians age of onset is more precocious in men than women [147].

The underlying mechanisms of sex-gender differences are still unknown. Most likely, cultural factors, education or social status linked with poorer access to health care services and self-care practices, and different lifestyle between genders or among ethnic populations, may contribute to such sex-gender differences in prevalence/incidence of diabetic neuropathy.

The autonomic neuropathy in type 2 diabetes depends on changes in sympathetic innervations, disordered adrenergic receptor expression, and altered catecholamine levels in the myocardium and manifests clinically as resting tachycardia, orthostatic hypotension, exercise intolerance, and silent myocardial infarction [148]. No clear data has been obtained about possible sex-gender differences in incidence rate of diabetic autonomic neuropathy. A recent study, concerning the prevalence of cardiac autonomic neuropathy (CAN) in a cohort of newly diagnosed diabetic patients has evidenced that it can be detected very early in type 2 diabetes reaching a rate of about 16% at the diagnosis [149]. This study, however, recruited a population prevalently made of men, although the design did not allow whether this really mirrored a real male prevalence in the early diagnosis of this complication. In addition, CAN partly contributes to induce QT prolongation which is the result of the total duration of ventricular myocardial depolarization and repolarization a parameter largely regulated by sexual hormones [150-152]. When this ECG parameter is corrected for heart rate (QTc), it is predictive of cardiovascular mortality in apparently healthy people of both sexes in non diabetic as well as in diabetic populations [153]. The prevalence of prolonged QTc interval, however is higher in people with type 1 and type 2 diabetes as compared to non-diabetic subjects and prolonged QTc is reported to be an independent marker for coronary heart disease in diabetes, having been demonstrated to be a highly significant predictor of cardiac death even in newly diagnosed type 2 diabetic patients [154]. Finally, the prolongation of QTc interval is assumed to increase the risk for development of malignant ventricular arrhythmias and has been demonstrated to be a highly significant predictor of cardiac death [155,156]. The cumulative incidence of prolonged QTc in type 1 diabetes has been found significantly different in men (13.9% versus women (24.5%), even after adjustment for confounding factors, such as age, BMI, physical activity, and blood pressure [157]. This

difference could justify the higher risk of cardiovascular disease and congestive heart failure observed in postmenopausal diabetic women with respect to diabetic men [22].

Nonetheless, according to a meta-analysis including 4,584 patients, 92% with type 1 diabetes, showed that at a 86% specificity prolonged QTc was a relatively more accurate indicator of autonomic failure in men than in women [158]. Autonomic diabetic neuropathy is often associated with hypoglycemic unawareness, a condition by which the subject does not feel the hypoglycemic state. This effect might increase the severity of the hypoglycemic events, is more frequent in women than in men, and in some situations might drive acute cardiovascular events such as stroke or myocardial infarction [159].

The urogenital localization of diabetic neuropathy in men is the cause of erectile dysfunction with a prevalence up to 50% in type 1 diabetes and up to 30 % in type 2 diabetes [160,161], while a more neglected aspect regards the female gender. In this regard only recently a paper highlighted the presence in diabetes of a neglected manifestation of urogenital female sensory neuropathy characterized by vulvar pain disorders or vulvodynia [162]. This should stimulate further research in this field, and should lead to add diabetes mellitus to the list of causes of vulvar pain disorders in future classifications possibly identifying the disease as 'diabetic vulvopathy' to better describe this clinical manifestation of diabetic neuropathy.

## CONCLUSIONS

This review confirms that important sex-gender differences are present in diabetes, especially in relation to macro-microvascular complications, as summarized in Table 2, rewritten from the frame of Table 1, in the perspective of specifically dealing with sex and gender differences in diabetes and its complications.

| Sex | Gender |
|---|---|
| • Anatomical differences *(vulovodynia, increased genital infections after SGLT2-inhibitors therapy)* | • Social position *(gender difference regards to interference with census and heath service opportunities)* |
| • Hormonal milieu *(increased CVD risk in diabetic women after menopause as compared to men, sex-driven modulation of diabetic renal disease with increased risk among men)* | • Occupation *(gender difference regards to the interference with physical activity or with health service opportunities)* |
| • Pregnancy *(gestational diabetes, increase in attributable risk associated with diabetes in women with previous gestational diabetes)* | • Education *(gender difference regards to educational factors related to interference with health service opportunities and habits)* |
| • Weight/body composition *(differences in BMI at diagnosis of diabetes, different fat disposition according to sex)* | • Physical activity *(gender differences in effects of physical activity on aging and with both primary and secondary prevention of diabetes and of its complications)* |
| • Differences in renal function *(modulation of susceptibility to diabetic nephropathy, differences in pharmacodynamics and kinetics)* | • Habits *(different impact by gender of smoking, alcohol intake, as related to the incidence of diabetic complications)* |
| • Differences in drug action *(sex-related differences in drug-kinetics,- dynamics and side effects)* | • Access to health service opportunities *(gender associated differences leading to late or evenly constant access to specialists, gender difference in prediction of CVD by adherence as to guidelines to diabetes process indicators)* |
| • Differences in life expectancy and in aging *(more prevalence rate of CVD, as first-ever events, recurrences and related disabilities in women of advanced age)* | • Different representation of gender in RCTs recruitment *(under-representation of women especially regarding RCTs of drugs prescribed for cardiovascular protection)* |
| • Blood biochemistry *(increased prevalence of atherogenic dyslipidemia, hypercoagulability, raised oxidative stress among diabetic women)* | • Influence by physician's gender in medical procedures |
| • Genetic predisposition/heredity to CVDs/neoplasms *(increased predisposition of myocardial infarction and ischemic stroke among diabetic women as compared to the male counterpart)* | • Gender difference in disease symptoms/signs awareness *(gender difference in perception of symptoms of major cardiovascular diseases associated with diabetic macroangiopathy)* |

**Table 2 –** Specific characteristics associated to sex- gender differences in diabetes and its complications (see text).

In summary, from this review it appears evident that if diabetes is a known risk factor for cardiovascular diseases for both genders, women are, however at a higher risk, especially in postmenopausal period. Since genetic risk loci for CVD are more readily detectable in women, more studies are needed to evaluate the real impact of genetic burden in explaining all sex-gender differences in diabetes associated cardiovascular diseases. Microvascular complications of diabetes are similarly sex-gender differentiated, but the differences are less well defined than in the macrovascular context. The lack of our capacity to identify directly the mechanism initiating the disease, instead of the epiphenomenon, is the cause of the partial failure in the control of diabetic microvascular complication.

An important aspect we did not consider in this review, for obvious limitation of space, is the sex-gender of the care-giver in respect of the patient. Indeed since women are considered with less risk and tend to reach less ambitious targets than men [163], it would be possible to speculate that, generally speaking, a male doctor might be more prone to consider a woman with less attention than a man, giving to the latter a more aggressive pharmacological attention. Another example of care-giver sex-gender difference might come considering for example the diabetic neuropathy: are there difference approaching the problem of male impotence if the doctor is a male or a female? In a female patient would a male diabetes care-giver face the possibility of the emerging problem of vulvodynia? These peculiar, even psychological, aspects of sex-gender differences should deserve more attention.

In conclusion all these differences should be altogether taken into account in order to address preventive and therapeutic strategies as properly and timely as possible, to reduce the impact of diabetes on both the survival and the quality of life of diabetic patients especially among women.

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