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Importance of macroprolactinemia in hyperprolactinemia

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Condensation

The presence of elevated monomeric prolactin concentrations indicates the requirement for further examinations and treatment with dopamine receptor agonists in patients with macroprolactinemia.

Abstract

Macroprolactin is an antigen-antibody complex of a higher prolactin (PRL) molecular mass (big-big, MW > 150 kDa), consisting of monomeric PRL and immunoglobulin G isotype. The term macroprolactinemia is suspected for cases when the concentration of macroprolactin exceeds 60% of the total serum PRL concentration determined by the polyethylene-glycol precipitation method. The gold standard of the diagnosis of macroprolactinemia is gel filtration chromatography. The prevalence of macroprolactinemia in hyperprolactinemic populations varies between 15 and 35%. Although the pathogenesis of these antibodies is not clear, it is possible that some changes in the pituitary PRL molecule represent an increased antigenicity to the immune system leading to the production of anti-PRL antibodies. A mild hyperprolactinemia usually occurs because macroprolactin is not readily cleared from circulation due to its higher molecular weight. Moreover, the hypothalamic negative feedback mechanism by autoantibody-bound PRL is inactive because macroprolactin cannot access to the hypothalamus and therefore results in hyperprolactinemia. Reduced *in vivo* bioactivity of macroprolactin may be the reason for the lack of hyperprolactinemic symptoms. It also seems that anti-PRL autoantibodies may compete with the PRL molecule for receptor-binding thus resulting in low bioactivity. Additionally, the large molecular size of macroprolactin confined in the intravascular compartment prevents its passage through the capillary endothelium to the target cell, which may be the reason for the absence of the symptoms. According to current concepts macroprolactinemia is considered a benign clinical condition in patients with with normal concentrations of bioactive monomeric PRL and with a lack or low incidence of hyperprolactinemic symptoms and negative pituitary imaging. In such cases, with resistance to antiprolactinemic drugs, no pharmacological treatment, diagnostic investigations or prolonged follow-up are required. However, macroprolactinemia may also occur in patients with any of the conventional symptoms of hyperprolactinemia which could not be differentiated from those with true hyperprolactinemia. These symptoms are mostly attributed to the excess of monomeric PRL and its presence is of overridden concern, and the diagnosis of macroprolactinemia is misleading

and inappropriate. A multitude of causes, like physiological, pharmacological and pathological, including stress, prolactinomas, hypothyroidism, renal and hepatic failure, intercostal nerve stimulation, and polycystic ovary disease can contribute to the rise in the levels of monomeric PRL. It is important for patients with elevated monomeric PRL to undergo routine evaluation of hyperprolactinemia identifying the exact pathologic state and introducing adequate treatment regardless of the presence of macroprolactin. In addition, macroprolactinemia may rarely occur due to macroprolactin associated with pituitary adenomas, with biological activity of macroprolactin comparable with that of monomeric PRL. In these cases when excess of macroprolactin occurs with clinical manifestations of hyperprolactinemia, macroprolactinemia should be regarded a pathological biochemical variant of hyperprolactinemia. An individualized approach to the management of such patients with macroprolactinemia may be necessary and pituitary imaging, dopamine treatment, and prolonged follow-up should be applied.

Keywords: Macroprolactin, Hyperprolactinemia, Diagnosis, Treatment

1. Introduction

Prolactin (PRL) is a single globular polypeptide hormone synthesized and secreted by pituitary lactotroph cells. Heterogeneity in its molecular size has been described in the serum and three major variants can be found including monomeric PRL, dimeric and polymeric isoforms. PRL is synthesized as a prehormone (MW 26 kDa) and after cleavage the resulting hormone is a monomeric isoform of PRL (little PRL, MW 23 kDa). This monomeric PRL is the major form in the blood of subjects with normoprolactinemia and true hyperprolactinemia, accounting for 80-95% of the total PRL. It is known to be both biologically and immunologically active *in vivo* with a half-life of 26-47 min. The other forms mainly include the dimeric (big PRL, MW 48-56 kDa) and the polymeric isoform (big-big PRL, MW > 150 Da) or macroprolactin. In normal sera the dimeric isoform makes up < 10% of the PRL present and the polymeric isoform accounts for a small (less than 1%) but variable percentage of the total PRL and these two forms are known to have lower biological activity. The term macroprolactinemia is characterized by predominant presence of macroprolactin and it is suspected mainly in asymptomatic subjects or those without the typical hyperprolactinemia-related symptoms. In addition to prolactinomas and neuroleptics/antipsychotic agents, macroprolactinemia is one the three most common causes of hyperproplactinemia [1]. The nature of macroprolactin is heterogenous and it is mainly identified as an antigen-antibody complex of high stability consisting primarily of monomeric PRL and immunoglobulin (Ig) G isotype. However, non-IgG-bound forms of macroprolactin (complexes with IgA or IgM; highly glycosylated monomeric PRL, covalent or noncovalent aggregates of monomeric PRL) are rarely detected, mainly in sera with marginally elevated levels. In spite of low *in vitro* bioactivity, the complex apparently lacks *in vivo* bioactivity although macroprolactin retains its immunoreactivity properties. Owing to its high molecular weight, macroprolactin is confined to the vascular system which may reduce its access to the PRL receptors of target organs in the periphery as well as centrally and it is thus bio-unavailable resulting in asymptomatic hyperprolactinemia [2,3]. Therefore, typical symptoms of hyperprolactinemia (oligomenorrhea, amenorrhea, galactorrhea,

infertility etc.) and abnormal imaging changes in the pituitary gland are not common in patients in whom macroprolactin is the predominant form of PRL. These IgG type autoantibodies have a low affinity and high capacity, and long-term follow-up revealed that macroprolactinaemia might be a long-lasting condition [3,4]. In patients with macroprolactinemia and normal concentrations of monomeric PRL there was a low incidence of hyperprolactinemia-related symptoms during prolonged follow-up. It has been suggested that macroprolactinemia should be considered a benign variant with mildly elevated PRL levels and a cause of evident resistance to antiprolactinemic drugs. Moreover, such patients can be reassured because no pituitary imaging investigations, dopamine agonist treatments, and prolonged follow-up would be necessary [5]. In addition, routine screening of all hyperprolactinemic sera for macroprolactin may be recommended because reduced use of imaging and dopamine agonist treatment in macroprolactinemic patients would be cost-effective resulting in net cost savings [6].

However, not all patients with macroprolactinemia lack clinical symptoms because there are a number of reports regarding the overlapping of the main hyperprolactinemic symptoms due to the rise in the levels of monomeric PRL in subjects with true hyperprolactinemia and those with macroprolactinemia [7-11]. Moreover, no laboratory features in addition to clinical features could reliably differentiate macroprolactinemic patients from those with monomeric hyperprolactinemia [8]. Comparison of multiple methods for the identification of hyperprolactinemia in the presence of macroprolactin revealed no difference in the prevalence of abnormal menses, galactorrhea, or abnormal pituitary imaging between the patients with and without macroprolactin [9]. Although oligomenorrhea and galactorrhea occurred more frequently in patients with true hyperprolactinemia, they also occurred in 57% and 29%, respectively, of macroprolactinemic patients and these differences were not sufficient to distinguish between the two groups on the basis of clinical symptoms alone. Moreover, hyperprolactinemia due to macroprolactin led to diagnostic confusion, unnecessary investigations, and unsuitable treatment before the introduction of macroprolactin screening by application of a reference interval to polyethylene glycol (PEG)-treated

hyperprolactinemic sera [10]. Therefore, it is important that laboratories introduce screening programmes to examine samples with elevated total immunoreactive PRL for the presence of macroprolactin and to determine the monomeric PRL component which is responsible for bioactivity *in vivo* [11]. In a recent study a few cases of macroprolactinemia of pituitary origin associated with prolactinoma experienced similar clinical manifestations comparable with that of monomeric hyperprolactinemia, and their disappearance after treatment with dopamine agonists suggesting bioactivity of macroprolactin. Therefore, in spite of the fact that macroprolactinemia had been considered a benign condition, pituitary imaging and conservative treatment with dopamine agonists and prolonged follow-up should be applied in these rare cases as well as in macroprolactinemic patients with elevated monomeric hyperprolactinemia [12]. In this review we report various clinical features of macroprolactinemia explained through pathophysiologic mechanisms suggesting different approaches which would contribute to the improvement of proper identification and adequate management of such patients.

2. Prevalence

The proportion of macroprolactinemia in the general population has previously been reported at 0.2% in women and 0.02% in men [13]. However, macroprolactinemia may be more common, with a recently reported prevalence of 3.7% and no difference in prevalence between genders [14]. Because the reported proportion of macroprolactinemia in hyperprolactinemic populations is much higher in most studies and varies between 15 and 35% (mean prevalence 25%), macroprolactinemia is therefore considered a common finding in endocrinological practice [3, 8, 12, 13]. One study reported a prevalence of 46%, but it is likely that this particular incidence reflected selection bias because of the specialized nature of the study center, which received samples sent from other laboratories when the possible diagnosis of macroprolactinemia was raised [15].

3. Clinical features

The earliest reports of macroprolactinemia were isolated cases in patients being investigated for nonreproductive endocrine problems or healthy research volunteers who had no symptoms of hyperprolactinemia, but normal menstruation and maintained fertility. Analysis of circulating PRL by column chromatography revealed that between 85-90% of serum PRL was big-big PRL with normal levels of monomeric PRL. The reduced biological activity of macroprolactin has been suggested as the reason for the lack of symptoms which might be the result of the big-big PRL complex preventing passage through the capillary endothelium to the target cell [16-18]. The new term macroprolactinemia was used for the first time in 1985 to denote a nonprogressive state and a novel cause of hyperprolactinemia [18].

However, further, more comprehensive serial investigations of patients with macroprolactinemia reported a mild symptomatology with a lower incidence of clinical symptoms and abnormal imaging findings in the pituitarry gland than in patients with true hyperprolactinemia [3, 5, 10, 15, 19-22]. During prolonged follow-up of a cohort of 51 patients with macroprolactinemia headache had been experienced in 12 patients (24%), oligomenorrhea in five (10%) and galactorrhea was present in only two cases (4%) with no symptomatic progression in any of the patients [5]. Among 113 patients with hyperprolactinemia clinical symptoms were more likely to occur in individuals with true hyperprolactinemia than in patients with macroprolactinemia (90% vs.54%) [15]. Out of 55 patients with macroprolactinemia none of the patients had sustained amenorrhea, one had galactorrhea and eight have had oligomenorrhea at age bellow 40 years [19]. Menstrual abnormalities and galactorrhea occurred less frequently in 106 patients with macroprolactinemia compared with patients with true hyperprolactinemia [20]. The prevalence of oligomenorrhea and galactorrhea was significantly higher in 164 patients with true hyperprolactinemia than in 21 patients with macroprolactinemia (46 vs. 14 % and 30 vs. 5 %, respectively) [21]. Among 337 patients with hyperprolactinemia more patients in the macroprolactinemic group were asymptomatic compared to the monoprolactinemic group (30.2 vs.

12.0%). Compared to the macroprolactinemic group, the monoprolactinemic group had a higher frequency of galactorrhea (39.2 vs. 57.1%) and abnormal magnetic resonance imaging findings (65.3 vs. 81.1%). It was concluded that macroprolactinemia should be considered a pathological biochemical variant of hyperprolactinemia that may present with any of the conventional symptoms and radiological findings generally associated with elevated PRL levels [22].

Morever, in other investigations symptoms related to PRL excess were reported more frequently in subjects with macroprolactinemia. There was no difference in the frequency of clinical features between macroprolactinemic and true hyperprolactinemic patients. [7-9, 23]. In addition to overlapping in hyperprolactinemia-related symptoms between the groups there were no significant differences in serum gonadotrophins, estradiol or testosterone levels. Nevertheless, the patients with macroprolactinemia had a lower infertility rate (6.7% vs. 32.7%) and a greater percentage of normal magnetic resonance imaging of the pituitary gland (73.3% vs. 34.5%) than those with monomeric hyperprolactinemia. Therefore, it was suggested that routine laboratory screening with PEG for all hyperprolactinemic sera might prevent the unnecessary use of radiological techniques and medical treatment for hyperprolactinemic patients [23].

4. Diagnosis

For an accurate diagnosis of macroprolactinemia laboratory and radiological techniques are essential in the evaluation of hyperprolactinemia.

The gel filtration chromatography (GFC) is known as the gold standard or the reference assay for the separation of macroprolactin from mono- and dimeric PRL. Although the technique of GFC is accurate and reproducible, it is an expensive, time-consuming and labor intensive method which precludes its widespread use and prompted more diagnostic laboratories to develop nonchromatographic screening methods [20].

Separation of macroprolactin by immunoadsorption with Protein A (PA) and Protein G (PG), immobilized on Sepharose, is based on high affinity of the PA and PG for human IgG, which is a primary antibody to PRL in serum. Although this method exhibits acceptable precision and the recovery of standard preparation is satisfactory, pretreatment of sera with PA and PG may lead to a significant overestimation of monomeric PRL concentrations [24].

Separation of macroprolactin by means of ultrafiltration is based on the passage of PRL through the separating membrane which selectively retains particles according to their molecular size, net charge and three-dimensional structure. Although ultrafiltration represents a practical and precise alternative to GFC for estimating the macroprolactin in serum, the PRL concentrations recorded after ultrafiltration, compared with those after GFC, may vary considerably from sample to sample [24, 25].

A number of studies have shown that PEG induced precipitation of macroprolactin in serum sample represents a simple, inexpensive, accessible, rapid and reproducible screening technique for hyperprolactinemic sera [19, 26, 27]. The method is based on different precipitation of proteins according to their molecular weight and solubility in different concentrations of PEG solution. To determine free PRL concentrations, a concentration of 25% PEG is added to the same volume of serum which precipitates out high-molecular weight constituents including immunoglobulins. It is not necessary to incubate the PEG-treated sample but the usage of cold PEG and the vortex mixing are important. Free PRL is defined as the PRL concentration in the supernatant after PEG precipitation, and total PRL is defined as the PRL concentration in the water-treated sample. The PEG–precipitable PRL (%) represents the amount of macroprolactin calculated as follows: (total PRL−free PRL)/total PRL×100. It has been generally accepted that a PEG-precipitation ratio greater than 60% (recovery less than 40%) is the cut-off value for the diagnosis of macroprolactinemia. This is because there is no a clear-cut value of “substantial increase”, but conventionally a diagnosis of macroprolactinemia is made when more than 30–60% of PRL is in

the macroprolactin form of GFC [6, 9, 24, 26-28]. Precipitation with PEG is a widely used screening test for macroprolactin and is easily performed in clinical laboratories. However, PEG also induces a partial precipitation of monomeric PRL (up to 25%) so reliance on the relative percentage of recovery lacks specificity which may lead to an underestimated evaluation and misinterpretation of actual PRL levels [24]. This is especially important in cases when an excessive macroprolactin occurs in patient's serum simultaneously with supraphysiological concentrations of monomeric PRL [9, 26, 29]. Furthermore, it has been reported that the presence of PEG in the sample can interfere with some PRL immunoassay procedures [30]. To avoid such problems, it has been recommended that each laboratory, undertaking macroprolactin screening, must establish method-specific reference intervals derived by use of PEG-treated sera from healthy individuals [9, 26, 29]. In routine laboratory diagnostics the post-PEG modified reference range is the best means to accurately identify patients with true hyperprolactinemia [9].

Data regarding radiological evaluation of the pituitary gland by using computerized axial tomography and magnetic resonance imaging are usually limited because the presence of macroprolactin is suspected mainly in subjects with mild hyperprolactinemia and negative pituitary imaging. The prevalence of macroprolactinemia among newly diagnosed prolactinoma patients is similar compared with the control group of healthy subjects (3.5 vs. 3.7%), and may be explained by the coexistence of a pituitary adenoma and macroprolactinemia or macroprolactin production by the pituitary tumour itself [12].

5. Pathophysiology

In the majority of patients the etiology of macroprolactinemia is thought to be an extrapituitary postsecretory phenomenon of anti-PRL autoantibodies, confined to the vascular system with most often lack of bioactivity *in vivo* and normal concentrations of monomeric PRL with the consequence being the absence of symptoms. This absence of macroprolactin in extravascular

spaces and the pituitary gland might be explained by the high molecular weight of the macroprolactin molecules, which cannot cross the endothelium and thus remain in the intravascular compartment preventing its access to PRL receptors at all. Because the clearance of macroprolactin by the kidneys is delayed macroprolactinemia may be a condition of prolonged duration. The clearance of PRL is slower in the presence of anti-PRL antibodies, suggesting that the antibody-bound PRL is big enough to be confined to vascular spaces, which also precludes filtration of the bound PRL filtration from the glomeruli. Therefore macroprolactinemia develops due to the delayed clearance of PRL rather than increased production. These PRL autoantibodies are stable with time and long-term follow-up (>10 years) revealed that macroprolactinemia might be a long-lasting condition in humans [3,4,19,28]. The second reason for the asymptomatic presentation of macroprolactinemia includes binding of anti-PRL autoantibodies to their binding sites (epitopes) in both N- and C-terminal residues of the PRL molecule which are located near the binding site 1 to PRL receptors. It seems that anti-PRL autoantibodies may compete with the PRL molecule for binding to its receptors, thus leading to low bioactivity *in vivo* [31]. Comparing two different bioassays it was found that the biological activity of human macroprolactin was considerably lower toward the homologous receptor-mediated Ba/F-LLP assay than in the commonly used heterologous rat Nb2 cell assay. Therefore, the usage of the homologous Ba/F-LLP assay correlates well with the assumption that the bioactivity of macroprolactin is very low *in vivo* and the activity displayed by macroprolactin toward the rat receptor may be inappropriate [32].

Although the pathogenesis and the source of these antibodies is not yet absolutely clear it is possible that some autoimmune disorders like thyroid disorders and systemic lupus erythematodes may be accompanied with macroprolactinemia [33]. However, other studies examining a large number of patients revealed no specific association between macroprolactinemia and systemic autoimmune disorders [20,34]. It was found that macroprolactinaemic sera did not yield evidence of an increase in markers of autoimmunity when compared with hyperprolactinaemic or normal sera [34]. It is proposed that genetic susceptibility and environmental factors are the mechanisms

involved in the development of anti-PRL autoantibodies which may alter the immune response in hosts as postulated in other autoimmune disorders [35]. On the other hand, pituitary PRL with some changes in its molecule represents an increased antigenicity to the immune system. It was found that phosphorylated forms of PRL were intolerant to the immune system and leakage of such forms upon hypophysitis or lack of dephosphorylation may cause an autoimmune response leading to the production of anti-PRL antibodies. The finding that IgG4 was a predominant subtype of PRL antibodies supports the possibility that it may be be produced by chronic antigen stimulation [36].

It has been suggested that anti-PRL autoantibody is a cause of hyperprolactinemia because a significant positive correlation may be found between anti-PRL autoantibody titers and serum PRL concentrations. It seems that the hypothalamic negative feedback mechanism by autoantibody-bound PRL does not work because the complex cannot access to the hypothalamus due its size and therefore results in mild hyperprolactinemia. However, when serum free PRL concentrations exceed normal PRL concentrations, negative feedback mechanisms begin operating to normalize free PRL levels which are usually in sera from most macroprolactinemic patients within normal range [14, 37]. Therefore the biochemical findings of serum estradiol and luteinizing hormone (LH) may be significantly higher in individuals with macroprolactinemia compared with those with true hyperprolactinemia [10, 18, 37].

Although the majority of macroprolactinemia cases are asymptomatic, a number of patients experienced the main hyperprolactinemia-related symptoms due to the rise in the levels of monomeric PRL, that could not be differentiated from those with true hyperprolactinemia [7-11]. The association of the relatively common symptoms of galactorrhea and oligomenorrhea in patients with macroprolactinemia characterized by the simultaneous presence of excess serum macroprolactin and monomeric PRL concentrations may be explained as coincidental [10]. In addition the macroprolactin complex may intermittently dissociate *in vivo* in some cases, releasing monomeric PRL that causes the symptoms attributable to hyperprolactinemia [38]. Moreover,

macroprolactinemia may occur simultaneously with increased concentrations of free PRL plus other causes of hyperprolactinemia. A range of other causes, from physiological (pregnancy), to pharmacological and pathological (including stress, hypothyroidism, renal and hepatic failure, intercostal nerve stimulation by trauma or surgery, and polycystic ovary disease) can contribute to a small rise in the levels of little PRL. The presence of increased levels of monomeric PRL is of primarly concern and therefore it is important in these symptomatic patients with macroprolactinemia to identify the exact pathologic state routinely and to introduce the adequate treatment with dopaminergic agonists. In cases of exclusion of all the causes and negative pituitary imaging idiopathic hyperprolactinemia should be established [39]. Moreover, it has recently been found that the coexistence of macroprolactinemia and the presence of raised free PRL concentrations in women with oligo-/amenorrhoea was associated with a higher risk of pituitary adenomas (36%). Therefore, pituitary magnetic resonance imaging should perhaps be mandatory in such cases [40]. However, hyperprolactinemia with high levels almost exclusively due to the presence of macroprolactin may occur in rare cases of invasive macroprolactinoma with preserved biological activity resulting in suppressed gonadotrophin levels and typical clinical presentation represents similarly to monomeric [12].

6. Conclusion

According to current concepts, macroprolactin is a biologically inactive, high molecular mass complex of PRL with IgG and its accumulation in serum has little, if any, pathological significance in patients with macroprolactinemia and normal concentrations of monomeric PRL. Its presence is suspected mainly in patients with mild hyperprolactinemia who are asymptomatic or with a low incidence of hyperprolactinemia-related symptoms and with negative pituitary imaging. Macroprolactinemia has been considered a benign clinical condition and thought to be a cause of apparent resistance to antiprolactinemic drugs. Therefore, no pharmacological treatment or other medical procedures and diagnostic investigations without prolonged follow-up may be required.

However, macroprolactinemia may also occur in patients with any of the conventional symptoms of hyperprolactinemia and radiological pituitary findings that could not be differentiated from those with true hyperprolactinemia. The symptoms of hyperprolactinemia are mostly attributed to the excess of monomeric PRL and therefore its presence is of overridden concern, and the diagnosis of macroprolactinemia in this setting is misleading and inappropriate. An excess of monomeric PRL with macroprolactinemia may be explained as coincidental or by intermittent dissociation of macroprolactin. In addition, a range of other causes, (physiological, pharmacological and pathological, including stress, pituitary adenomas, hypothyroidism, renal and hepatic failure, intercostal nerve stimulation, and polycystic ovary disease) can also contribute to the rise in the levels of monomeric PRL. It is important for patients with elevated monomeric PRL to undergo routine evaluation of hyperprolactinemia identifying the exact pathologic state and introducing adequate treatment regardless of the presence of macroprolactin. In addition, macroprolactinemia of pituitary origin with clinical manifestations similar to patients with true hyperprolactinemia may rarely occur due to bioactive macroprolactin associated with pituitary adenomas. In these cases when excess of macroprolactin occurs with clinical manifestations of hyperprolactinemia, macroprolactinemia should be regarded as a pathological biochemical variant of hyperprolactinemia. An individualized approach to the management of such patients with macroprolactinemia may be necessary and pituitary imaging, dopamine treatment, and prolonged follow-up should be applied. However, in cases of exclusion of all the causes and negative pituitary imaging idiopathic hyperprolactinemia should be established.

Therefore, it would be recommendable to make different professionals (such as endocrinologists, gynaecologists, pharmacologists and general practioners and all those involved in the management of hyperprolactinemia due to macroprolactin) aware of macroprolactinemia because of its frequent occurrence and timely recognition. In addition, for the accurate and early-stage diagnosis of macroprolactinemia a close communication between the laboratory and the clinicians should be more frequently applied. Moreover, the importance of routine screening of all

hyperprolactinemic sera for macroprolactin and a more meaningful clinical measurement of the bioactive monomeric PRL content with proper medical education and knowledge dissemination of the meaning of macroprolactinemia would be desirable.

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