Current Perspective

Macroprolactinemia: a New Cause of Hyperprolactinemia

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Abstract. Hyperprolactinemia is not only seen in pregnancy but also in several pathological conditions such as prolactin (PRL) secreting pituitary adenoma (prolactinoma), intracranial tumors compressing the pituitary stalk or hypothalamus, and PRL stimulative drugs. However, some patients with hyperprolactinemia are diagnosed as having idiopathic hyperprolactinemia because the causes are unknown. They are subjected to repeated radiological examinations to find a microadenoma, to a long-term treatment with bromocriptine, and even to a surgical intervention. There is accumulating evidence that macroprolactinemia, in which most circulating PRL forms large protein complexes (more than 150 kDa), is a major cause of idiopathic hyperprolactinemia. The patients with macroprolactinemia are clinically characterized by the lack of hyperprolactinemia-related symptoms such as amenorrhea and galactorrhea. We found that anti-PRL autoantibody is a leading cause of macroprolactinemia that might be heterogeneous in nature. Most patients with anti-PRL autoantibodies were symptom-free and pregnancy was possible despite a marked hyperprolactinemia. Identification of macroprolactinemia is clinically important to prevent unnecessary examinations and treatments in patients with idiopathic hyperprolactinemia.

Keywords: prolactin, autoantibody, macroprolactinemia, immunoglobulin G, hyperprolactinemia

I. History of macroprolactinemia

Human prolactin (PRL) is heterogeneous in molecular size: the major circulating form is little PRL (MW 23 kDa), the remainder consisting of big PRL (MW 50 kDa), and big-big PRL (MW greater than 150 kDa) (1, 2). However, some patients with hyperprolactinemia have a high proportion of big-big PRL in their serum (Fig. 1a). Whittaker et al. (3) first described an interesting case of hyperprolactinemia in whom the serum PRL was predominantly big-big PRL on gel chromatography, the clinical symptoms of hyperprolactinemia such as amenorrhea and galactorrhea were lacking, and spontaneous pregnancy was possible despite hyperprolactinemia. Jackson et al. (4) first used the new term 'macroprolactinemia' to describe a patient with marked hyperprolactinemia comparable with PRL secreting pituitary adenoma (prolactinoma) whose PRL mainly consisted of big-big PRL. Subsequently, several cases of macro-

prolactinemia have been reported (5 – 8). However, the pathogenesis of macroprolactinemia was unknown. We identified anti-PRL autoantibodies in some patients with idiopathic hyperprolactinemia (9, 10), and the clinical and biochemical features were quite similar to those of macroprolactinemia: serum PRL was mainly composed of big-big PRL, they generally lacked clinical symptoms of hyperprolactinemia, and spontaneous pregnancy occurred without bromocriptine treatment. Anti-PRL autoantibodies were considered to be major contributors to the cause of macroprolactinemia.

This new entity of hyperprolactinemia is clinically important because hyperprolactinemia due to macroprolactin has been classified as 'idiopathic' and repeated examinations by computerized axial tomography (CT) or magnetic resonance imaging (MRI) have been performed to pursue a possible microadenoma of the pituitary gland. These patients have also been treated with bromocriptine on a long-term basis. This review presents recent progress in the diagnosis and pathophysiological understanding of macroprolactinemia.

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II. Prevalence

The causes of hyperprolactinemia include pregnancy, prolactinoma, intracranial tumors compressing the pituitary stalk or hypothalamus, PRL stimulative drugs, hypothyroidism, chest wall diseases, and hepatorenal diseases. However, 8.5 – 40% of hyperprolactinemia is classified as 'idiopathic hyperprolactinemia' because the causes are unknown (11, 12). Macroprolactinemia is included in this category, and we found that 12 of 75 (16%) patients with idiopathic hyperprolactinemia had macroprolactinemia due to anti-PRL autoantibodies (13). Recently, the prevalence of macroprolactinemia was investigated in a large population using a polyethylene glycol method and/or gel chromatography. Bjøro et al. (14) examined 605 hyperprolactinemic sera in routine diagnosis and found that 157 sera (26%) had an increased level of macroprolactin. Leslie et al. (15) examined 1,225 hyperprolactinemic sera and found that a total of 322 of the patients (26%) had macroprolactinemia. Vallette-Kasic et al. (16) performed serum PRL chromatography in 368 among 1,106 hyperprolactinemic patients because of discordant clinical, biological, or neuroradiological findings. They found 106 patients with macroprolactinemia, and so the incidence of macroprolactinemia in hyperprolactinemic populations was at least 10%. Although these studies did not include the data on anti-PRL autoantibodies, it is most probable that most macroprolactinemia is attributed to anti-PRL autoantibodies. Lira et al. (17) also reported that 5 of 8 pregnant women with macroprolactinemia had anti-PRL autoantibodies.

Since requests for PRL determination in men are less than in women, sexual difference in the frequency of macroprolactinemia is difficult to evaluate. We reported 2 men with macroprolactinemia and another study showed the sex ratio (F/M) of macroprolactinemia to be 16/1 (16). Similarly, chances for PRL determination in children are rare but 4 girls with macroprolactinemia were reported (16).

III. Etiology

There are several reports suggesting that big PRL in the serum is derived from covalently or non-covalently bound little PRL to another serum component, possibly recently identified PRL-binding protein (PRLBP) (18, 19). As for the etiology of big-big PRL (macroprolactin), we (9, 10) and other investigators (17, 20, 21) found anti-PRL autoantibodies in macroprolactinemic sera. Supporting evidence is the finding of macroprolactin in the fetal cord blood from a mother with macroprolactinemia (7), suggesting the passive transfer

of immunoglobulin G (IgG). Autoantibodies to other hormones such as insulin (22) and thyroid hormone (23) are well documented. The antiinsulin autoantibodies are produced without previous insulin injection and occasionally cause hypoglycemic attack that can be explained by the release of insulin from the insulin-IgG complex. Antithyroid hormone autoantibodies have no such biological function but only cause falsely high immunoassay values by interference. We found that the proportion of macroprolactin separated by different methods varied: 15% by ^{125}I-PRL binding, 47.9% with a protein G column (affinity column for IgG), and 83.4% by a polyethylene glycol method (13). Leite et al. (20) demonstrated that 24 – 86% of macroprolactin reacted as immunoglobulin-bound PRL as determined by protein A sepharose column chromatography (affinity column for IgG). Cavaco et al. (21) found that an average of 60% of macroprolactin was retained by affinity chromatography with an antihuman IgG agarose column. These differences may be the results of methodological problems, that is, the autoantibodies can be associated with endogenous PRL more efficiently than radio-iodinated PRL or PRL may be dissociated from the affinity column for IgG during column operation, resulting in underestimation of IgG-bound PRL. However, the possibility that IgG non-associated components may be involved in the formation of macroprolactin cannot be excluded. We found a pregnant woman with macroprolactinemia who did not have anti-PRL autoantibodies (24). The molecular weight of her macroprolactin was 230 kDa and a significantly high proportion of PRL (41.1%) was adsorbed to a concanavalin A column (affinity column for glycosylated protein). Repetitive freezing and thawing of the isolated macroprolactin resulted in a partial conversion to big and little PRL, and reduction of the isolated macroprolactin with 2-mercaptoethanol almost completely converted macroprolactin to little PRL. These findings suggest that this woman had heterogeneous complexes of covalently or non-covalently bound form of PRL with increased glycosylation. Carlson et al. (8) also reported two patients with macroprolactinemia whose macroprolactin was an aggregate of 25-kDa glycosylated and 23-kDa non-glycosylated PRL.

IV. Clinical manifestations

Historically, the first patient with macroprolactinemia was described as having hyperprolactinemia due to macroprolactin but normal menstruation and maintained fertility (3). Jackson et al. (7) described two women with macroprolactinemia who had normal menses, minimal galactorrhea, and spontaneous conception. However, not

all patients with macroprolactinemia lack clinical symptoms of hyperprolactinemia. We studied 15 women with macroprolactinemia and found that 11 had normal menses, 2 had oligomenorrhea, and 2 were in post-menopause. Two patients complained of minimal galactorrhea (13). A patient with oligomenorrhea and minimal galactorrhea showed high PRL levels of both macroprolactin and little PRL, which may be the reason why this patient had symptoms of hyperprolactinemia. More recently, large-scale studies were performed. Leslie et al. (15) reported that among 55 patients with macroprolactinemia, none had sustained amenorrhea, eight had oligomenorrhea at age less than 40 years, and only one had galactorrhea. Hauache et al. (25) examined 52 patients with macroprolactinemia and found that 46% had no symptoms of hyperprolactinemia, whereas only 10% of the patients with hyperprolactinemia due to other causes were asymptomatic. Vallette-Kasic et al. (16) examined 106 patients with macroprolactinemia and found that 61% had normal menstruation and 54% did not have galactorrhea.

The reasons why most patients with macroprolactinemia lack clinical symptoms of hyperprolactinemia are unclear. We initially speculated that IgG-bound PRL would have lower bioactivity because some large forms of PRL reportedly had reduced biological activity (5) and anti-human PRL antibody raised against rabbits is known to inhibit PRL bioactivity in Nb2 rat lymphoma cells (26). However, we found that IgG-bound PRL had comparable biological activity to little PRL determined by the bioassay system (27). It is likely that the autoantibodies do not interfere the binding between PRL and its receptors due to the different binding sites or to their lower affinity (10, 13). Then, why do most patients with anti-PRL autoantibodies lack clinical symptoms of hyperprolactinemia in vivo, although the IgG-bound PRL was fully bioactive in vitro? One possibility is an abnormality in the PRL receptor and/or postreceptor signal transduction pathways. This is unlikely, however, from a clinical point of view. We found that when the anti-dopaminergic agent domperidone was administered in a patient with anti-PRL autoantibodies, little PRL levels increased and galactorrhea occurred. In addition, when patients with anti-PRL autoantibodies became pregnant, little PRL levels as well as macroprolactin levels increased, and normal physiological responses to hyperprolactinemia such as amenorrhea and lactation occurred in such patients (13). These findings suggest that a rise in little PRL caused the clinical symptoms of hyperprolactinemia, suggesting that the binding of PRL to the receptor and postreceptor signal mechanism are intact. Another possibility is that IgG-bound PRL cannot exert its full biological activity in vivo because the access of PRL to target cells through the capillary wall may be restricted because of the large molecular size and/or changes in net charges.

V. Laboratory diagnosis

Diagnosis of macroprolactinemia is made mainly by two methods, a polyethylene glycol method and gel chromatography. Polyethylene glycol (PEG), a material used in radioimmunoassay (RIA) for precipitating hormone and its antibody complex, has been widely used to identify the presence of macroprolactin in the serum. Macroprolactin, if present, is precipitated with 12.5% PEG, leaving decreased PRL values in the supernatant. Recovery of less than 40% is taken as evidence that a significant level of macroprolactin is present in the serum (15). This is a simple and inexpensive test that can easily be integrated into laboratory practice. Gel filtration chromatography of the serum followed by PRL determination in each fraction has also been used for detecting macroprolactin, although it is time-consuming and expensive compared with the PEG method (16). The above two methods are used to identify the presence or absence of macroprolactin. To characterize the nature of macroprolactin, we performed an ^{125}I-PRL binding study, in which serum samples were incubated with ^{125}I-PRL followed by 12.5% PEG treatment to precipitate bound ^{125}I-PRL. We found that the patients with macroprolactinemia had increased ^{125}I-PRL binding, suggesting that binding components with large molecular weight existed in the serum of these patients (13). We also demonstrated that the large molecule that binds PRL is IgG by using a protein G column. Protein G binds to only IgG and its subclasses, separating out IgA, IgM, IgD, and albumin, which may bind to other affinity gels such as protein A. We found that 47.9% of serum PRL was bound to protein G in patients with macroprolactinemia, suggesting that almost half of the macroprolactin is IgG-bound PRL (Fig. 1b) (13). Concanavalin A is used to identify glycosylated PRL that occasionally forms another type of macroprolactinemia (24).

VI. Effects of macroprolactin on serum PRL measurements

It is well known that anti-thyroid hormone autoantibodies interfere with radioimmunoassay (RIA) of thyroid hormone and cause falsely high and low results with the double and single antibody technique, respectively (23). We, therefore, examined the effects of anti-PRL autoantibodies on serum PRL measurements by single antibody RIA, double antibody RIA, and immu-
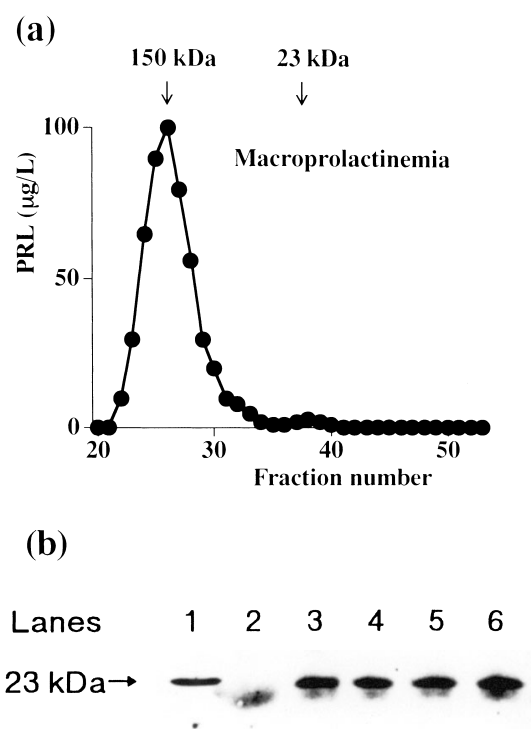
(a)

(b)

Fig. 1. Immunoreactive PRL in patients with anti-PRL auto-antibodies detected by gel chromatography and Western blotting. a: Representative gel filtration profile of immunoreactive PRL in patients with anti-PRL autoantibodies. PRL was not eluted at the 23-kDa authentic position but at greater than 150 kDa. (Revised from Ref. 9). b: Western blot of PRL in IgG fractions in patients with anti-PRL autoantibodies. IgG was purified from sera using a protein G column, and PRL contained in the IgG fraction was immunoprecipitated with rabbit anti-human PRL antiserum plus goat anti-rabbit IgG antiserum. SDS-PAGE without 2-mercaptoethanol, followed by Western blotting using anti-human PRL antiserum, was performed. The bands were detected by chemiluminescence. PRL was dissociated from IgG under the nonreducing condition and immunostained at the same position as the 23-kDa PRL standard (lane 1) in patients with anti-PRL autoantibodies (lanes 3 – 6). The 23-kDa PRL band was not observed when IgG from a patient with prolactinoma was used (lane 2). (Revised from Ref. 27).

noradiometric assay (IRMA) (28). Both single and double antibody RIA yielded lower PRL values than those by IRMA. IgG purified from serum with anti-PRL autoantibodies dose-dependently decreased the recovery of PRL assayed by double antibody RIA, while it did not affect that by IRMA. When a single antibody RIA in which bound tracer is nonspecifically precipitated by PEG is used in the presence of anti-PRL autoantibodies, the levels of radioactivity in the precipitate increase, resulting in lower values. Since thyroid hormone is a small molecule, the autoantibodies and reagent anti-bodies may recognize the same antigenic site. Therefore, binding of ^{125}I-thyroid hormone to the reagent antibody may be partly displaced by the autoantibodies, the

second antibody recognizes the first reagent antibody, not human IgG, and the radioactivity in the precipitate decreases, resulting in higher values in double antibody RIA. On the other hand, PRL is a polypeptide possessing several antigenic sites and therefore may be accessible both to endogenous autoantibodies and the reagent antibody. Since the second antibody may cross-react somewhat with human IgG, a larger amount of ^{125}I-PRL is precipitated, causing falsely low results. In IRMA, radiolabeled antibody sandwiches PRL with the solid phase antibody. Because PRL has multi-antigenic sites, these reagent antibodies may recognize anti-PRL auto-antibody-bound PRL as well as free PRL in a similar way. However, more recent studies indicate that PRL values in sera containing macroprolactin varied greatly using different commercial immunoassay kits (29), suggesting that anti-PRL autoantibodies may mask some epitopes of the PRL molecule, which are also the target of some reagent antibodies.

VII. Pituitary imaging

Since macroprolactinemia has been classified as idio-pathic hyperprolactinemia, pituitary imaging by CT or MRI is basically negative. However, we found a patient with prolactinoma and macroprolactinemia (13). Leslie et al. (15) reported that pituitary imaging was normal in 51 of 55 (92.8%) patients with macroprolactinemia. Vallette-Kasic et al. (16) reported that pituitary MRI was found to be normal in 63 of 81 (78%) patients with macroprolactinemia. They found an intrasellar arachnoidocele in 8 patients, a microadenoma in 3, a macroadenoma in 2, and an intrasellar pituitary cyst in 5 patients. Hauache et al. (25) reported that normal pitu-itary images were found in 41 of 52 (78.8%) patients with macroprolactinemia. Because of the recent progress in the quality of CT and MRI, the presence of abnormal-ities in the pituitary gland such as incidentaloma has been increasingly recognized. Aron et al. (30) reported that 10% of healthy subjects had radiographic evidence of a pituitary adenoma. Therefore, it is not surprising that some patients with macroprolactinemia possess some radiographic abnormalities in the pituitary gland. However, the frequency of positive imaging is much less in patients with macroprolactinemia than in those with hyperprolactinemia due to other causes.

VIII. Mechanism of hyperprolactinemia due to anti-PRL autoantibodies

Hyperprolactinemia higher than 200 μg/L is thought to be pathognomonic of pituitary adenoma in non-pregnant women (31). However, some patients with

anti-PRL autoantibodies had PRL values greater than 200 μg/L. As we pointed out in the previous section, hyperprolactinemia in patients with anti-PRL autoantibodies is not caused by interference with the assay system but actually reflects elevated serum PRL values. We confirmed this by showing that the serum was also hyperprolactinemic by an Nb2 bioassay system, which yielded similar values to those by immunoassay (27). In addition, we observed high levels of total PRL extracted from serum by acidification (10).

The causes of hyperprolactinemia in patients with anti-PRL autoantibodies are uncertain but several possibilities are worth considering. First, bound PRL is probably prevented from glomerular filtration, which is a major site for elimination. Second, since autoantibodies may compete with the receptors for PRL binding and bound-PRL is less likely to interact with PRL receptors than free PRL, it also partially escapes degradation in target organs. Third, bound PRL may not play an autoregulatory role in its own secretion at the pituitary and/or hypothalamus, and hypersecretion of PRL continues until free PRL reaches a certain level. The above possibilities are all based on the assumption that because of its large size and/or changes in net charge, the access of autoantibody-bound PRL through the capillary wall may be restricted. We found that there was a positive correlation between the titers of anti-PRL autoantibodies and the serum PRL levels (Fig. 2), suggesting that the autoantibodies are the cause of hyperprolactinemia. We also found that the PRL suppressive effects of dopamine and bromocriptine were delayed and incomplete in patients with anti-PRL autoantibodies (Fig. 3) (13).

Moreover, we demonstrated that the autoantibody-bound PRL was cleared from the circulation more slowly than free PRL (Fig. 4) (27). These findings support the hypothesis that hyperprolactinemia in patients with anti-PRL autoantibodies is due to delayed clearance of the autoantibody-bound PRL.

IX. Natural history of macroprolactinemia

A long-term follow-up (3 – 5 years) could be obtained in 4 patients with anti-PRL autoantibodies (13). Hyperprolactinemia persisted during these periods and two of
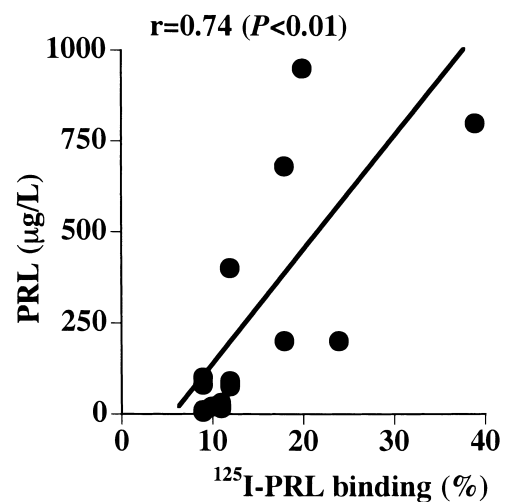
Fig. 2. Relationship between the titers of anti-PRL autoantibodies and the serum PRL levels. There was a significant positive correlation between them (r = 0.74, $P<0.01$). (Revised from Ref. 13).
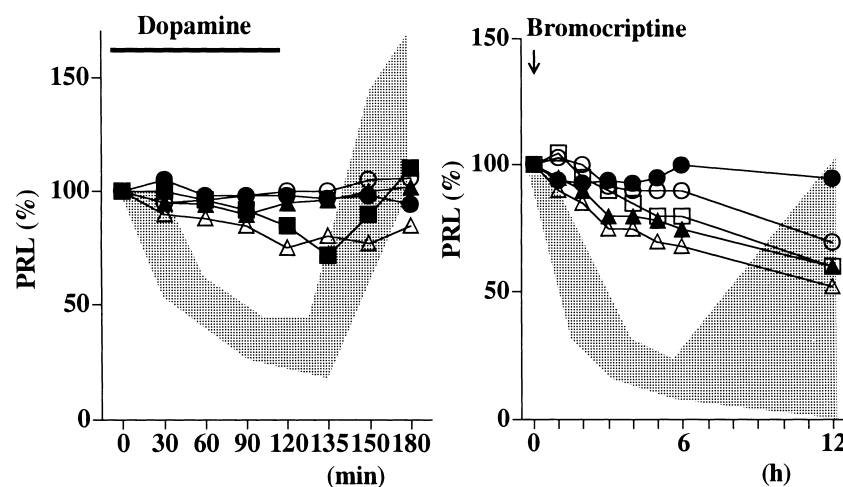
Fig. 3. PRL responses to dopamine (2 μg/kg/min, div) and bromocriptine (2.5 mg, p.o.) in patients with anti-PRL autoantibodies. The results are presented as percentage of the basal PRL levels. Dotted areas represent the mean ± 2S.D. in patients with prolactinoma. PRL suppressive effects by dopamine and bromocriptine were delayed and incomplete in patients with anti-PRL autoantibodies. (Revised from Ref. 13).
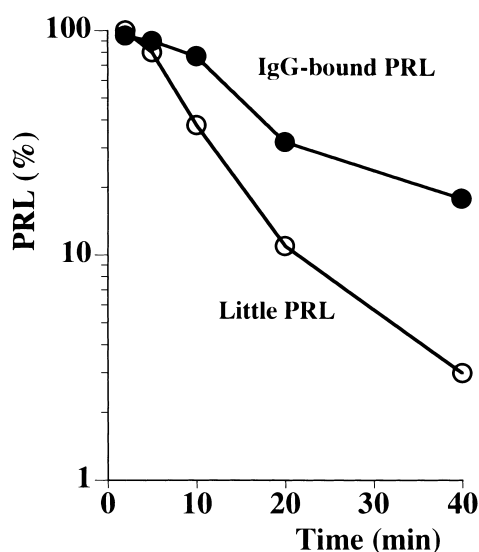
Fig. 4. Disappearance curves of immunoreactive PRL from the plasma when anti-PRL autoantibody bound PRL (closed circle) or PRL standard mixed with normal IgG (open circle) was injected intravenously into anesthetized rats. The anti-PRL autoantibody-bound PRL was cleared more slowly than free PRL. (Revised from Ref. 27).

Fig. 5. The changes in the gel filtration profiles of PRL during pregnancy (open circle: first trimester, closed triangle: second trimester, closed circle: third trimester) in a patient with anti-PRL autoantibodies. As pregnancy progressed, all three species of PRL increased, but the relative proportion of macroprolactin decreased. (Revised from Ref. 24).

them became pregnant without bromocriptine treatment despite marked hyperprolactinemia (200 – 300 μg/L). The serum PRL concentrations began to increase during the first trimester and peaked at term with extremely high levels (PRL more than 1000 μg/L) and normal lactation started after delivery in both patients. This finding is in good agreement with the previous report by Whittaker et al. (3). We examined the changes in the gel filtration profile of PRL during pregnancy (Fig. 5) (24). In the first trimester, most immunoreactive PRL was macroprolactin (90%). As pregnancy progressed, all three forms of PRL increased but the relative proportion of macroprolactin decreased (63.6%) in the third trimester. The release of little PRL from the pituitary gland may increase as pregnancy advances, and some little PRL may bind to anti-PRL autoantibodies, leading to an increase in the macroprolactin levels. However, when the levels of little PRL exceed the binding capacity of the autoantibodies, little PRL may start to increase. The titers of anti-PRL autoantibodies were not significantly changed in any of them during these periods. Recently, Vallette-Kasic et al. (16) reported a long-term follow-up in 42 patients with macroprolctinemia including 23 who were followed-up for 5 years or more. They reported that the PRL concentration remained grossly stable over the follow-up period.
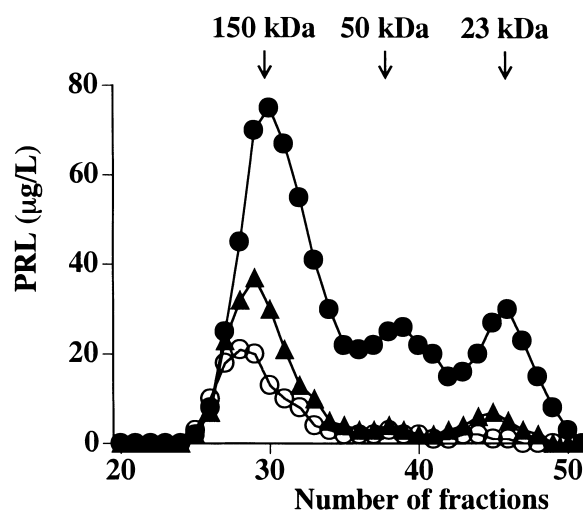
X. Concluding remarks

Macroprolactinemia has recently been increasingly recognized by a simple PEG method. The presence or absence of macroprolactin should first be screened in all hyperprolactinemic serum samples. If the patients with a positive PEG test lack clinical symptoms of hyperprolactinemia such as amenorrhea and galactorrhea, it is most likely that the hyperprolactinemia is due to macroprolactin. Repeated hormone or neuroradiological examinations and unnecessary treatments with bromocriptine should be avoided. Macroprolactinemia, a new cause of hyperprolactinemia, should be noted not only among endocrinologists, gynecologists, and pharmacologists but also among general practitioners.

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