Study of anti-Müllerian hormone levels in patients with Myotonic Dystrophy Type 1. Preliminary results

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Myotonic dystrophy type 1 is a multisystemic disorder characterized by myotonia, muscle weakness and involvement of several organs and apparatus such as heart, lungs, eye, brain and endocrine system. Hypogonadism and reproductive abnormalities are frequently reported. A progressive testicular atrophy occurs in about 80% in the affected males leading to Leydig cell hyperplasia and elevated basal follicle stimulating hormone (FSH) levels. Anti-Müllerian hormone (AMH) – a dimeric glycoprotein belonging to the super-family of transforming growth factor beta (TGF-beta) – is the earliest Sertoli cell hormone secreted in males and, together with inhibin B and FSH, is an important indicator of Sertoli cell function. AMH levels remain high during the whole prepubertal phase and are down-regulated in puberty by the increasing testosterone levels. Aims of the work were to assess the AMH levels in 50 patients with Myotonic Dystrophy type 1 aged less than 50 years and to investigate whether it may contribute to the endocrine function impairment observed in these patients. The results confirmed a reduction of testosterone levels associated with an increase in Luteinizing Hormone (LH) and FSH compared to controls, suggesting a reduced function of the Sertoli cells. Conversely the average levels of AMH were significantly lower in patients compared with controls, and almost undetectable in about 60% of them. Further studies are necessary to better clarify these findings.

Key-words: myotonic dystrophy type 1, gonadal function, anti-Müllerian hormone

Introduction

Myotonic dystrophy type 1 (DM1) or Steinert disease is a multisystemic disorder characterized by myotonia, muscle and facial weakness, cataract, cognitive and gastrointestinal involvement, and cardiac conduction abnormalities (1). DM1 is the most common adult muscular dystrophy with a global incidence of 1:8000. Symptoms appear between 20 and 40 years of age and the localization of muscle weakness is predominantly distal (1-4). It is a RNA-mediated disease caused by a trinucleotide expansion, the CTG repeat in the DMPK gene (4) on the long arm of chromosome 19 (19q13-2). The endocrine system is also frequently involved as hypogonadism and reproductive abnormalities (1-4). Progressive testicular atrophy is a prominent feature and occurs with an incidence of 80% in the affected males (5). The observed histological abnormalities include tubular atrophy, hyalinization and fibrosis of seminiferous tubules as far as a reduced sperm number. Oligo/azoospermia is reported in 73% of DM1 patients while low serum testosterone levels are observed in most patients. The progression of the disease leads to Leydig cell hyperplasia, elevated basal follicle stimulating hormone (FSH) levels and gonadal dysfunction (6-13). Therefore the evaluation of gonadal function, including interstitial Leydig cells and tubular Sertoli cell hormone production, is recommended in the workup of male hypogonadism.

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein composed of two 72 KDa monomers belonging to the super-family of transforming growth factor beta (TGF-beta) (14). The coding gene for this hormone is localized in humans on the short arm of chromosome 19.
(19p13.3). The gene spans 275 bp and is subdivided into five exons. The expression of AMH in males is usually restricted to foetal and post-natal testosterone cells, and, in females, in post-natal granulosa cells.

The AMH molecule takes its name from the first function described in foetal sex differentiation, the regression of Müller ducts in the early phase of male differentiation. In the male foetus the Sertoli cells secrete AMH and androgens. Androgens in turn stimulate the evolution of Wolff’s ducts into the male genital apparatus, while AMH causes the irreversible regression of the Müller’s ducts, which is completed at the end of the ninth week of gestation. With the exception of a transient decrease in the perinatal period, the testicular secretion of AMH remains at high levels until puberty (Fig. 1) (14-16). For such behaviour, AMH dosage was proposed as a marker for the evaluation of Sertoli cell activity and an early identification of the pre-puberal male hypogonadism (17-23).

The study aimed at evaluating the gonadal function of patients with Myotonic Dystrophy type 1, and the possible involvement of the AMH in mechanisms underlying the hypogonadism and reproductive abnormalities frequently observed in these patients. The purpose was to highlight a possible association between testosterone, LH, FSH and estradiol levels with those of AMH, and to investigate a possible correlation between AMH levels and age of patients and/or degree of CTG triplet expansion.

Subjects and methods

Fifty male patients affected by Myotonic Dystrophy type 1 aged between 18 and 50 years, regularly followed at the Cardiomyology and Medical Genetics of the “L. Vanvitelli” University, and 60 age-matched adult males were consecutively enrolled in the study.

![Figure 1](image.png)

**Figure 1.** Schematic representation of serum levels of gonadotropins (FSH and LH), testosterone (T), inhibin B (Inh B) and AMH from birth through adulthood (left axis) and percentage of Sertoli cells expressing the androgen receptor (AR, right axis).

All patients had the clinical diagnosis confirmed by molecular analysis to define the magnitude of the triplet expansion. In practice, on the occasion of the routine follow up, a blood sample was taken for hormone (testosterone, 17ß-estradiol, luteinizing and stimulating follicle hormones) dosage, while an aliquot of the collected serum was used to dose the AMH. A written informed consent was obtained from all participants to the study, that was approved by the local ethical committee.

The hormone dosage was performed according to CLIA Method DiaSorin, while AMH was dosed by a II generation ELISA kit (Beckman Coulter, Brea, CA, USA).

**Statistical analysis**

The values are shown as mean ± SEM. Statistical differences were analysed by Student t test for non paired data; significance was put for p values < 0.05.

**Results**

The results are summarized in Table I and Figure 2. Serum testosterone levels – although within the normal ranges – were on average significantly lower in DM1 than in controls (272.4 ± 55.8 ng/dL vs 563.3 ± 80.74 ng/dL, p value 0.0083; Fig. 2A).

The average values of LH and FSH were above the maximum limit of normality and statistically higher than in controls (11.1 ± 2.9 UI/L vs 4.4 ± 0.8 UI/L, p value < 0.0425, and 19.4 ± 4.7 UI/L vs 6.0 ± 1.4 UI/L, p value < 0.013, respectively; Fig. 2B-C).

Conversely, the average values of estradiol were not significantly different compared to controls (27.2 ± 3.7 pg/ml vs 30.5 ± 5.0 pg/ml, p value 0.6; Fig. 2D).

Focusing on AMH levels, a broad spectrum of values was observed in patients with DM1, ranging from 0.01 to 8.5 ng/ml. Furthermore 59% of them showed almost undetectable values (0.11 ± 0.07 ng/ml, p value 0.0064). The mean value of the whole group was 2.4 ± 1.4 ng/ml, significantly lower compared with controls (6.4 ± 1.4 ng/ml, p value 0.0318; Fig. 2E).

To be noted that patients who had almost undetectable values of AMH, presented also the highest values of LH and FSH (14.4 ± 4.3 UI/L vs 3.7 ± 0.95 ng/ml and 24.9 ± 5.6 vs 5.3 ± 1.3 UI/L), and the differences were statistically significant (p value 0.042, 0.006 and 0.006 respectively; Fig. 2F).

A trend to a negative correlation between AMH levels and age of patients and size of triplet expansion were observed (see Figures 3A and 3B, respectively).
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Discussion

Myotonic Dystrophy type 1 is a multisystem disease, with a wide pattern of clinical manifestations. Among these, the alterations of the endocrine system and in particular hypogonadism is one of the most frequently observed feature. The evaluation of the gonadal function, including interstitial Leydig cells and tubular Sertoli cells hormone production, is therefore recommended in these patients by the routine investigation of serum levels of testosterone, LH, FSH, and estradiol. As AMH has recently shown to play an important role in development of gonads and testicular function, and indicated as a possible marker of spermatogenesis, the dosage of serum AMH levels is also recommended. The evaluation of the gonadal function confirmed a condition of hypogonadism in our population, as serum testosterone levels were significantly lower compared to controls tough within the normal ranges. “However an impairment of the endocrine function and in particular of the Sertoli cells can be hypothesized by the observation that LH and FSH mean levels are statistically higher compared with controls.

Interestingly, it was observed that the average levels of AMH were significantly lower in patients compared with controls, and almost undetectable in about 60% of them. A trend to an inverse correlation between AMH and FSH was observed as the lower AMH levels were, the higher the levels of FSH. Further investigation are necessary to better define the contribution of the AMH in the impairment of the endocrine function in these patients, as far as that of proteins recently shown to be implicated in spermatogenesis (25-28).

Acknowledgements

The financial support of the Department of Experimental Medicine of University of Campania “Luigi Van-

| Table I. Hormonal values in patients with Myotonic Dystrophy type 1. |
|---------------------------------|-----------------|-----------------|-----------------|
| Serum hormones                  | DM1 patients    | Controls        | P value         |
| Testosterone (ng/dL)            | 272,4 ± 55,8    | 563,3 ± 80,7    | 0,0083          |
| LH (UI/L)                       | 11,1 ± 2,9      | 4,4 ± 0,8       | 0,0425          |
| FSH (UI/L)                      | 19,4 ± 4,7      | 6,0 ± 1,4       | 0,013           |
| Estradiol (pg/ml)               | 27,2 ± 3,7      | 30,5 ± 5,0      | 0,6             |
| AMH (ng/ml)                     | 2,4 ±1,0        | 6,4 ±1,4        | 0,0318          |

Figure 2. Hormone levels in DM1 patients and controls (A-F).
Testosterone levels (A); LH and FSH (B,C); Estradiol (D); AMH levels (E). Comparison of AMH and LH and FSH in the 2 groups (F).

Figure 3. (A) AMH mean values according to the age of patients; (B). AMH mean values according to the number of triplets.
“vitelli” is gratefully acknowledged. We thank patients and their families for the cooperation.

References