THE EFFECT OF TESTOSTERONE DEPRIVATION ON ATHEROPLAQUE FORMATION, TESTOSTERONE RECEPTORS, AND COLLAGENIZATION IN WISTAR PENILE TISSUE

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ABSTRAK

Tujuan Penelitian: *Mengevaluasi hubungan antara deprivasi testosteron dengan pembentukan ateroplak, reseptor testosteron dan kolagenisasi jaringan penis Wistar.* ***Bahan & Cara:*** *Enam belas ekor Wistar dibagi menjadi dua kelompok, masing – masing delapan ekor, yakni kelompok orkidektomi dan kelompok kontrol yang menjalani prosedur sham. Dua bulan setelah orkidektomi, dilakukan pemeriksaan patologi anatomi jaringan kavernosa penis dari kedua kelompok. Untuk deteksi ateroplak dipakai pewarnaan Hematoxyllin-Eosin, dan untuk deteksi reseptor testosteron dipakai pewarnaan imunohistokimia, sedangkan untuk deteksi fibrosis dipakai pewarnaan kolagenisasi. Perbedaan pembentukan ateroplak, ekspresi reseptor testosteron, dan kolagenisasi dari kedua kelompok dinilai dengan menggunakan uji Chi kuadrat, Fischer, dan uji t independen.* ***Hasil Penelitian:*** *Ditemukan perbedaan bermakna terhadap ekspresi reseptor testosteron dan kolagenisasi dari kedua kelompok, sedangkan dalam pembentukan ateroplak tidak ditemukan perbedaan bermakna.* ***Simpulan:*** *Deprivasi testosteron seperti pada disfungsi ereksi akibat Late-Onset Hypogonadism menyebabkan perubahan arsitektur jaringan erektil.*

Kata kunci: *Disfungsi ereksi, reseptor testosteron, kolagenisasi, pembentukan ateroplak, orkidektomi.*

ABSTRACT

Objective: *To evaluate the association between testosterone deprivation and atheroplaque formation, testosterone receptors, and collagenization in Wistar penile tissues.* ***Material & method:*** *Two months after orchiectomized bilaterally, penile tissue of eight Wistar were harvested for semi-quantitative analysis of atheroplaque formation using hematoxyllin-eosin staining. Similar analysis were also conducted to look for collagenization by collagenase staining as well as the expression of testosterone receptors by immunohistochemistry staining. Control group were eight Wistar underwent Sham surgery. Statistical analysis for atheroplaque formation in Wistar compare to control group used was Chi-square, Fishers' exact test was used for the expression of testosterone receptors, and independent t-tests for evaluating collagenization.* ***Results:*** *A significant decline in the testosterone receptors and increase collagenization were obtained in orchiectomized Wistar, while atheroplaque formation was not significantly different.* ***Conclusion:*** *Testosterone deprivation revealed a significant decline in the expression of testosterone receptors, and a significant increase in collagenization while atheroplaque formation was not significantly changed.*

Keywords: *Erectile dysfunction, testosterone receptors, collagenization, atheroplaque formation, orchiectomy.*

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BACKGROUND

Erectile dysfunction (ED) is a prominent global health problem in aging male. Several studies have revealed a staggering prevalence that ED encompasses 35% of men aged 60 years or above.[1] The multiple etiologic factors imply that either systemic and local changes that affect innervations, blood supply and endothelial of the penis would ultimately results in ED.[2-7]

Sexual hormones were thought to play critical role in the failure of erectile mechanism, either

directly or indirectly by inducing changes toward organic ED.[8-13]

The precise mechanism through which testosterone deprivation causes ED is still unclear,[12] but the critical role of testosterone in maintaining the microarchitecture of penile tissue has been described.[9] Testosterone has bee noted to exert protective effect from systemic and local atheroplaque formation.[4,12]

One of the mechanism through which testosterone exerts its effects in penile tissue is interaction with testosterone receptors, forming ligand-receptor complex that further initiates cascade of reaction in penile tissue.[1,14] Such ligand-receptor process is most prominent in early ages when the penis has not reached its maximum growth. However, the existence and function of testosterone receptor in adult penis is still debated. Several studies on animal model showed that testosterone deprivation resulted in decreased intracavernosal pressure and penile vascular endothelial degeneration.[9] Testosterone deprivation achieved by orchiectomy was associated with tissue atrophy, apoptosis and smooth muscle fibrosis that ultimately lead to venogenic ED, and the landmark of degeneration is collagenization of penile tissue.[1,3,8]

Results from several studies showed that a part of ED population would exhibit only marginal response,

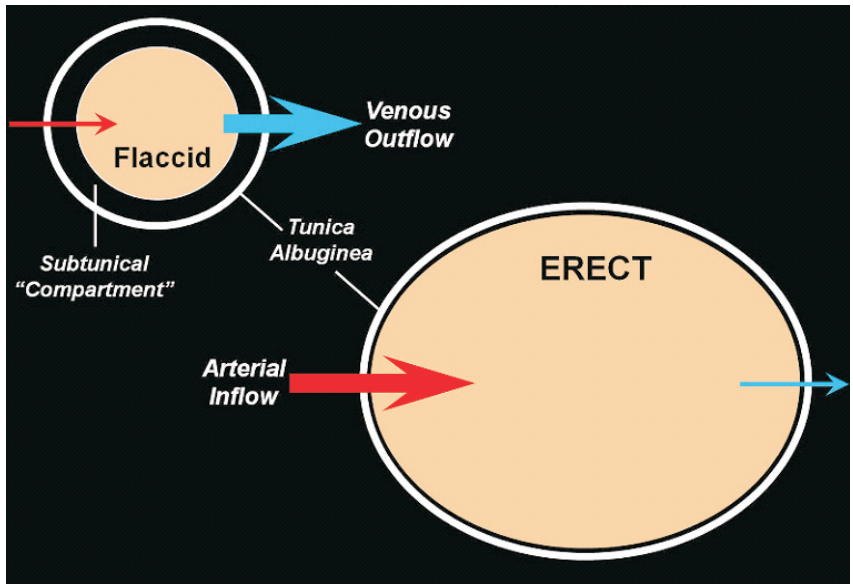


Figure 1. Schematics of venooclusion mechanism of erection[2]

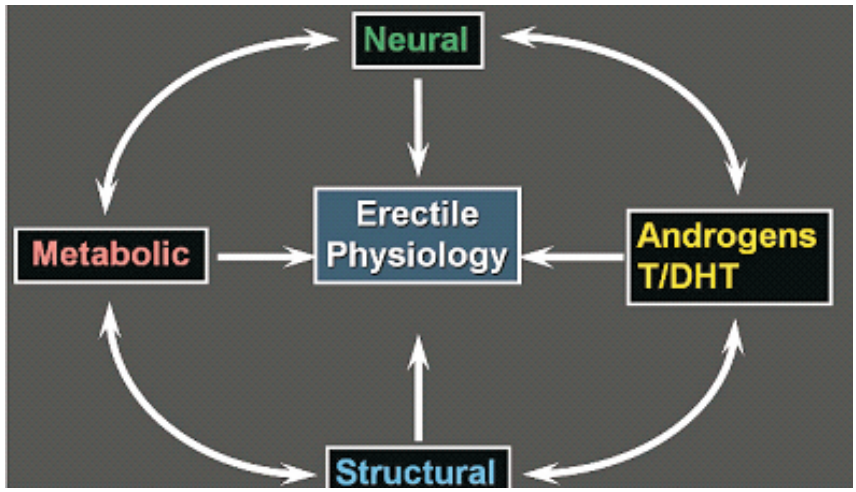


Figure 2. Proposed multifactor interaction in erectile physiology[2]

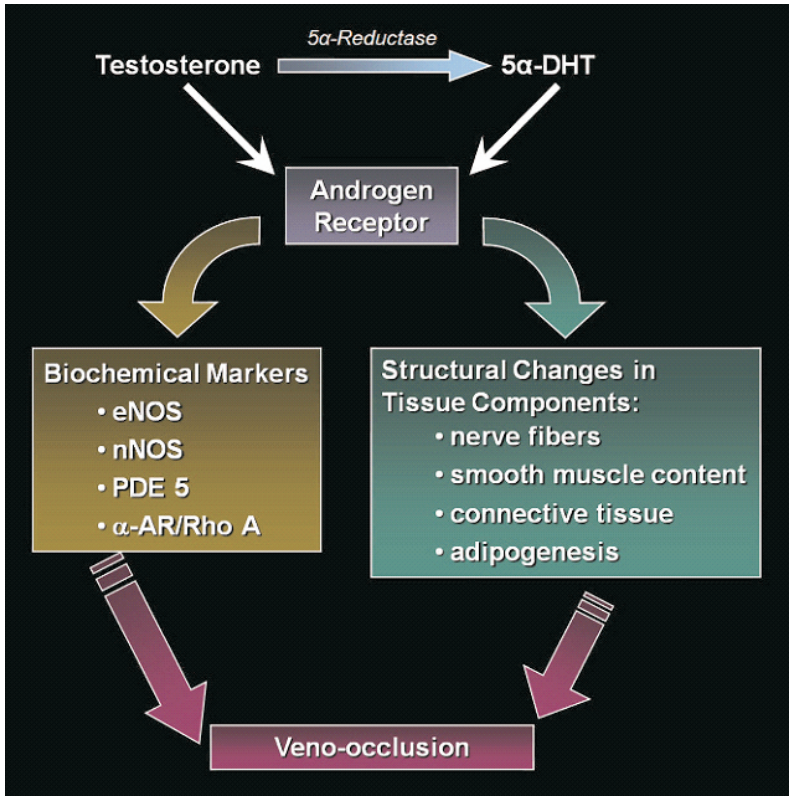


Figure 3. Role of testosterone in venooclusion mechanism of erection[2]

or even refractory to PDE5i treatment.[11,12] The fact of refractory ED and additional data of testosterone deprivation in penile tissue has triggered several theories on possibility of the benefits from testosterone supplementation in ED treatment.[11-15]

OBJECTIVE

To investigate the correlation between testosterone deprivation with atheroplaque formation, testosterone receptor expression, and cavernosal collagenization.

MATERIAL & METHOD

The study was conducted from May to August 2010. Study subjects were comprised of 16 wistar rats weighed between 300-400 grams and equally assigned to control (n = 8) and treatment group (n = 8). All rats were declared healthy by corresponding veterinarian and those who died during study period were replaced and subjected to all procedures of treatment protocol of the study.

The control group underwent sham operation whereas treatment group underwent bilateral orchidectomy using external extraction method as described previously by van Praag.

After the procedure, the rats were kept in cage for eight weeks before terminated humanely using lethal dose of intravenous ketamine. Subsequently, all groups were subjected to penectomy, and penile tissues were then obtained for further histopathological and immunohistochemistry staining for analysis using CX20 Olympus microscope by a pathologist.

Results from the pathologist were then analysed statistically using student t-test for parametric data and chi square for categorical data. Statistic calculation was performed using SPSS 16 for Windows software with $p < 0,05$ considered significant.

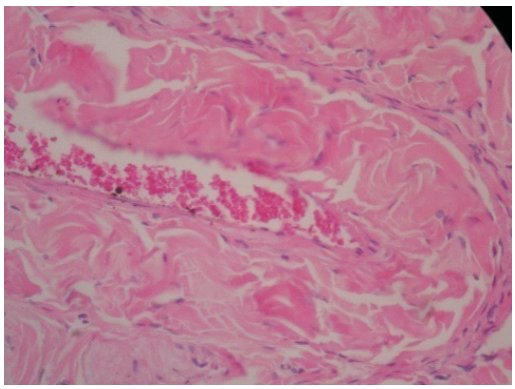
RESULTS

There were one atheroplaque formations found in each control and treatment group. This finding is considered not significant ($p = 1,00$).

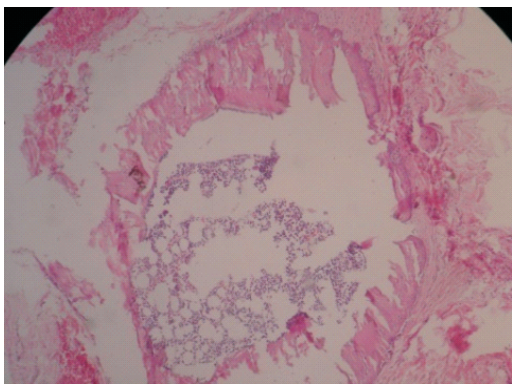
Table 1. Atheroplaque formation.

Group	Atheroplaque	
	Negative	Positive
Control	**7**	1
Treatment	**7**	1
Total	14	2

(p = 1,0)



(A) Atheroplaque



(B) No atheroplaque formation

Figure 4. Atheroplaque

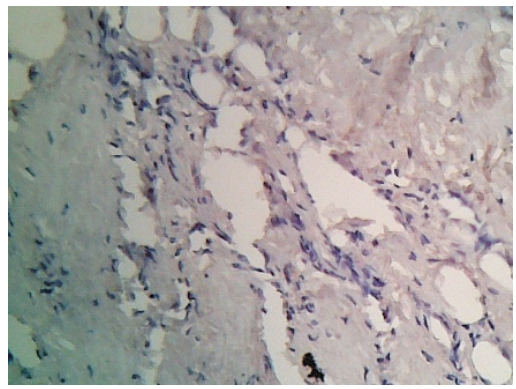
Testosterone receptor expression is defined as brownish appearance of the nuclei in immunohisto-chemistry staining. Bilateral orchidectomy showed sig-nificant effect on testosterone receptor. There were six rats in treatment group that have been found without testosterone receptor in their penile tissue (p = 0,02).

Using collagenase staining, collagenization of the specimens were confirmed by comparing the percentage of collagenization in four field of viewing in 400x magnification.

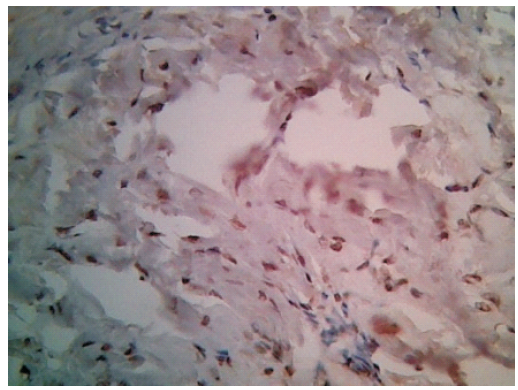
Table 2. Testosterone receptor.

	Positive	Negative	Total
Orchiectomy	2 (4,5)	6 (3,5)	8
Control	7 (4,5)	1 (3,5)	8
Total	9	7	16

(p = 0,02)



(A) Negative testosterone receptor



(B) Positive testosterone receptor

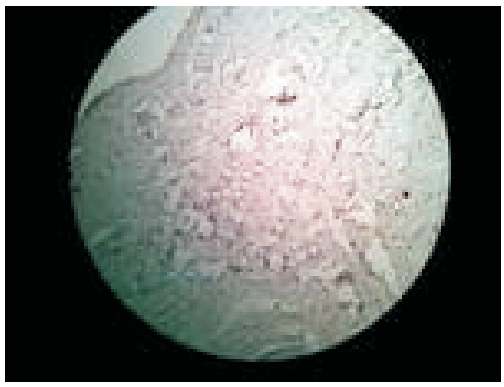
Figure 5. Testosterone receptors.

All rats in treatment group showed high-intensity collagenization (100%), whereas control group exhibit only mild-to moderate-intensity collagenization (p = 0,02).

Table 3. Cavernosal collagenization.

Group	High Intensity	Moderate Intensity	Low Intensity
Castrated	4 (100%)	0 (0%)	0 (0%)
Control	0 (0%)	2 (50%)	2 (50%)

(A) High intensity collagenization



(B) Moderate intensity collagenization

(C) Low Intensity collagenization

Figure 6. Cavernosal collagenization.

DISCUSSION

Penile tissue changes that occurred after orchidectomy the critical role of testosterone in maintaining the penile microstructural integrity and thus, its normal functions, especially those related to the erection venooclusion mechanism. It has been previously proposed that disorders of erectile mechanism is an interrelated multifactor process. Currently the evidence for our study would factor the contribution of testosterone receptor expression and collagenization toward the entire spectrum of organic ED, especially venogenic ED.

CONCLUSION

Bilateral orchiectomy results in penile tissue microarchitecture change, namely the increase of collagen content in corpus spongiosum and the decrease of testosterone receptor expression. Regardless of its multifactor origin, mechanisms by which testosterone deprivation leads to ED need more precise elaboration, since this would contribute in proposing the benefit of testosterone supplementation in ED cases.

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