Correlations of the Mechanisms Involved in Diabetes Mellitus, Peripheral Arterial Disease and Gangrene Leading to Amputations - a Retrospective Study Over the Non-traumatic Amputations Performed in the Emergency Clinical County Hospital of Arad Between 2014-2018

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Diabetes mellitus (DM) is, undoubtedly, an important risk factor of peripheral artery disease (PAD), leading to increased and severe complications, as well as for primary or associated dermatological lesions, all of which leading to radical therapeutic measures, as amputations are. DM and its complications have an important effect on life expectancy and quality of life, which can be quantified using disability-adjusted life year (DALY) as universal metric that allows researchers and policymakers to compare very different populations and health conditions across time. In the last five years these medical conditions have had a detrimental effect on the whole Arad County society, with a magnitude of premature deaths (YLL) of 1,779 years and a value for Years Lost due to Disability (YLD) of 20,795, DALY reaching 22,574. These figures have to change completely the existent DM control measures for better protection of patients' health and improving their quality of life.

Keywords: Diabetes mellitus, amputations, DALY

Diabetes mellitus (DM), peripheral arterial disease (PAD) and gangrene are complications which can lead to extreme surgical procedures such as amputations, that are irreversible, mutilating, and strongly affecting quality of life determinants as much as some cancers do [1].

DM selectively damages endothelial and mesangial cells, whose glucose transport rates do not decline rapidly as a result of hyperglycemia, leading to high glucose inside the cell [2]. There are four main mechanisms involved in diabetic's cells' distructions: the first is the polyol pathway and increased polyol pathway ûux, described in peripheral nerve [3], with an effect on decreasing nerve conduction velocity in the DM patients. The second mechanism was described by increased formation of advanced glycation end products (AGEs) [4], these AGE precursors can diffuse out of the cell modifying extracellular matrix molecules nearby, causing cellular dysfunction by modifying circulating proteins in the blood such as albumin, proteins which can then bind to AGE receptors and activate them, thereby causing the production of inûammatory cytokines and growth factors, which in turn cause vascular pathology. The third pathway: hyperglycemia-induced activation of protein kinase C (PKC) isoforms [5]; many abnormal vascular and cellular processes, including endothelial dysfunction, vascular permeability, angiogenesis, cell growth and apoptosis, changes in vessel dilatation, basement membrane thickening and extracellular matrix expansion, enzymatic activity alterations such as mitogen-activated protein kinase (MAPK), cytosolic phospholipase A2 (PLA2), Na+–K+–ATPase and alterations in several transcription factors, are attributed to multiple PKC isoforms that are changed by DM [6]. The fourth mechanism: increased hexosamine pathway flux and consequent overmodiûcation of proteins by N-acetylglucosamine are followed by a high concentration of glucose which may lead to glucosamine formation, with consequences in Nitric oxide-mediated dilatation of arterioles [6].

PAD is listed as one of the diseases derived from atherosclerosis, so its understanding should be focus on the pathophysiological role played by inûammation [7], which is fundamental connected both in triggering PAD and in augmenting it. The endothelium is highly to endowed in nitric oxide generation (NO) and its release. Because NO release lowers endothelial barrier damag; since endothelial dysfunction is considered an early signal of atherosclerosis, reduction in NO bioactivity as well as the increased generation of oxygen free radicals (or reactive oxygen species ROS) are effective players in endothelial dysfunction [8].

C-reactive protein (CRP), an acute-phase protein of hepatic origin, inhibits the release of NO, promotes the release of endothelial monocyte chemo-attractor protein-1 (MCP-1) and tissue factor. It is strongly associated with the increased risk of atherosclerotic cardiovascular disease independent of cholesterol level [9-12].

Interleukin-6 GG genotype contributes to the development of PADs among individuals with Type 2 DM [13]. IL-1 contributes in PAD development to extending inflammatory activity, leading to cell proliferation (i.e., neutrophils) [14]. A shortfall in IL-10 has been associated to increased atherosclerotic plaque [15].

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Selectin (S) is a family of three closely related glycoproteins [14]. P-Selectin is highly involved in the atherosclerotic process affecting platelet aggregation, promoting the up-regulation of tissue factor which plays a crucial role in determining arterial thrombosis [15]. Increased E-selectin plasma levels were found in PAD patients with Type 2 DM.

Matrix Metalloproteinases (MMPs) are a family of Zinc 2+ dependent enzymes involved in platelet aggregation, thus suggesting a possible role in the atherosclerotic process [8]. The results of MMP studies have shown that MMP 2 and 9 might be useful in marking macrovascular damage in PAD patients and in Type 2 DM [16-19].

Gangrene

It has been demonstrated that inhalation of O_2 at pressures greater than 1ATA (atmosphere absolute) as it happens in hyperbaric oxygen therapy, increase production of ROS, which are in the same time signaling molecules in transduction cascades or pathways for a variety of growth factors, cytokines [16], and hormonal substances, with a beneficial role in chronic non-healing leg-diabetic wounds, associated with vascular insufficiency due to accelerated atherosclerotic process [17,20].

We studied upper and lower-extremity nontraumatic amputations performed between 2014-2018 in Arad County, taking into account DM complications, PAD and dermatological related lesions, focused on burden of disease as is reflected in disability-adjusted life years (DALY) and Kaplan-Meier estimation for hospital stay. This subject was partially treated before [21], without PAD as main contributor.

Aim

Identification of all nontraumatic upper and lower-extremity amputations in relation to DM, PAD and skin disorders in Arad inpatients between 2014-2018.

Hypotheses

The alternative hypothesis: there is more risks for nontraumatic amputations in PAD and DM patients compared to dermatological ones. The null hypothesis: all patients are at the same risks for nontraumatic amputation.

Experimental part

Material and methods

Electronic hospital database was scanned for all DRG (Diagnosis Related Groups) cases as listed in Romanian Tables of RO DRG related to upper and lower-extremity amputations as well as nontraumatic diseases codes identified as causes for these procedures. A retrospective chart review of medical records for all inpatients was the basis for a descriptive epidemiological study which was performed by calculating DALY according to Arad County population registered in the official demographic records [22,23]. Years of Life Lost due to premature mortality (YLL) was calculated by totalling the number of deaths at each age between 1-79 years for women and between 1-71.6 years for men, multiplied by the number of years of life remaining up to the age of 79 years for women and 71.6 for men, according to the Life Expectancy tables [24]. Univariate analyses were used to identify significant differences in variables for inpatients. Chi square testing was destinated to compare intergroup variation between nonparametric variables; logistic regression model was designed to evaluate independent associations between demographics, and significant clinical variables identified from univariate analyses. Odds ratios (OR) and 95% confidence intervals were calculated for each variable in the regression models. Statistics were performed with *IBM SPSS® Statistics 24*, *MedCalc®* and *Epi Info® 7*.

Results and discutions

In total ten thousand eight hundred seventy-five (10,875) patients were assisted in the hospital; 6,439 for DM; 4,101 for dermatological conditions (of which 1,188 presented both) and 335 for PAD. Inpatients age-categories and death

Age category	survival	deceased	total	cases to 10,000	death rate to 10,000
aged 0-4	101	0	101	48.97	0
aged 5-9	44	0	44	18.95	0
aged 10-14	79	0	79	34.32	0
aged 15-19	120	0	120	48.39	0
aged 20-24	75	0	75	27.66	0
aged 25-29	88	0	88	24.09	0
aged 30-34	104	0	104	31.03	0
aged 35-39	159	1	160	40.88	0.26
aged 40-44	324	0	324	82.01	0.00
aged 45-49	577	5	582	141.34	1.21
aged 50-54	918	7	925	356.26	2.70
aged 55-59	1,603	17	1,620	507.52	5.33
aged 60-64	1,741	32	1,773	561.43	10.13
aged 65-69	1,606	28	1,634	626.22	10.73
aged 70-74	1,202	44	1,246	785.62	27.74
aged 75-79	1,074	27	1,101	673.89	16.53
aged 80-84	621	23	644	624.82	22.31
85 and over	236	19	255	352.06	26.23
total	10,092	203	10,276	360.40	7.12

Table 1
INPATIENTS AGE CATEGORIES WITH PREVALENCE AND DEATH RATES TO 10.000

item	DM	Dermatology	PAD
sociodemographic			
mean age	62.35	59.16	68.83
SD	12.769	18.713	11.742
gender			
F	3586	2100	93
M	2853	2001	242
residence			
rural	3210	2097	159
urban	3229	2004	176
evolution			
progressive	28	2	10
improved	5555	3761	174
deceased	172	10	21
stationary	130	26	16
recovered	554	302	114
total cases	6439	4101	335
Death rate			
F	2.31	0.28	9.67
M	3.11	0.19	4.95
Mean hospital stay	8.24	7.91	10.11
procedures			
incision, excision and drainage	61	178	3
upper extremities amputation	11	0	0
lower extremities amputation	686	7	122
upper extremity amputation	11	0	0
arterial bypass and endarterectomy	5	0	4
abscess drainage	5	4	0
wound debridement	227	492	16
embolectomy	1	0	36
skin grafting	2	60	0
wound dressing	1	5	1
varicose vein treatment	0	15	0

Table 2
INPATIENTS CHARACTERISTICS

rates, as listed in table 1, shows that age-category prevalence was greater for those aged 70-74, deaths included.

Gender mean age was higher for women 62.98 years (n=5,779; Standard Deviation SD 15.233) and 59.49 years for men (n=5,096; SD 15.363). Gender ratio F:M was almost equal 1.13:1. Table 2. Death rate was 2.06% for M (n=105) and 1.69% (n=98) for F, with notable differences: for DM death rate was 3.11% in M and 2.31% in F; for dermatology was 0.19% for M and 0.28% for F. Interesting, in PAD death rate for women was 9.677% and for men was 4.95%. Amputation of lower limb rates in DM inpatients were 10.65% and 0.17% for upper limb. In dermatology amputation rate was 0.17% for lower limb. In PAD were met only lower extremities amputations at the highest rate of 36.41%.

Relative Risk (RR) for PAD is 2.9509 for men compared to women (95% CI 2.3302 to 3.7369, P < 0.0001) and chance (OR) for PAD is 3.0482 for men compared to women (95% CI 2.3926 to 3.8834, P < 0.0001). RR for DM is slightly higer 1.1084 for men compared to women (95% CI 2.3302 to 3.7369, P < 0.0001) and OR for DM is 1.2856 for men compared to women (95% CI 1.1907 to 1.3881, P < 0.0001). RR for dermatological diseases is 1.0806 for men compared to women (95% CI 1.0296 to 1.1340, P = 0.0017) and OR is 1.1327 for men compared to women (95% CI 1.0480 to 1.2241, P = 0.0017).

Upper and lower-extremity amputations were performed on patients between 38 - 95 years of age, to 233 women and 593 men, gender ration M:F being 2.54:1 (P < 0.0001). RR for amputation is 2.8862 for men compared to women (95% CI 2.4921 to 3.3425, P < 0.0001) and OR for amputation is 3.1346 for men compared to women (95% CI 2.6803 to 3.6658, P < 0.0001). RR for death after amputation is 2.3957 compared to conservative procedures (95% CI 1.7680 to 3.2461, P < 0.0001) and OR for death after amputation is 2.6965 compared to conservative procedures (95% CI 1.8675 to 3.8936, P < 0.0001). The death rate not related to amputation was 1.68%, while death rate related to amputation was 4.55%. Overall death rate was 1.87% with the highest value for PAD, 6.27%, table 3.

Mean survival according to hospital stay was 111.44 days for PAD (Standard Error SE 4.788), 72.69 for DM (SE 3.365) and 53.37 for dermatological inpatients, (SE 1.439) Figure 1, Kaplan Meier survival curve.

	Number of events		Number censored		
Factor	death	%death	N	%	Total sample size
PAD	21	6.27	314	93.73	335
dermatology	10	0.24	4,091	99.76	4,101
DM	172	2.67	6,267	97.33	6,439
Overall	203	1.87	10,672	98.13	10,875

Table 3
DEATH CASES SUMMARY

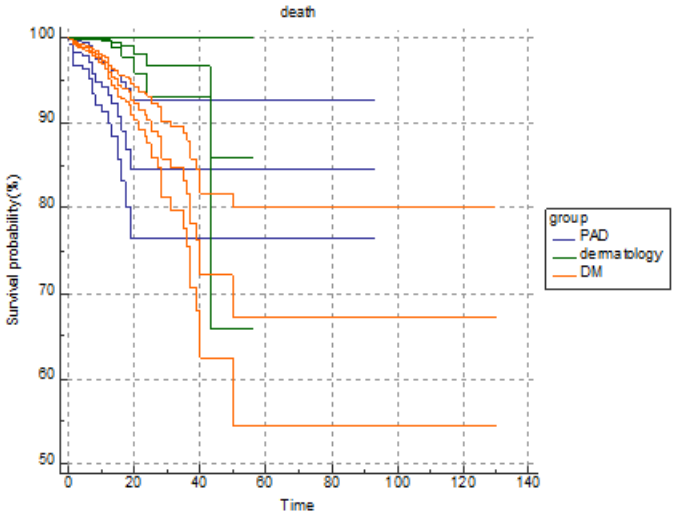

Fig.1. Kaplan Meier survival curve

Table 4

YLL FOR DM, PAD AND RELATED DERMATOLOGICAL CONDITIONS OVER 2014-2018

	Deaths	Deaths per 1,000	Av. Age at death	Standard LE	YLLs	YLL per 1,000
Males						
35-39	1	0.05	37.5	32.0	21	1.0
40-44	0	0.00	42.6	27.5		0.0
45-49	4	0.19	47.7	23.4	67	3.3
50-54	4	0.31	52.6	19.7	59	4.6
55-59	9	0.60	57.6	16.0	114	7.6
60-64	24	1.68	62.7	12.7	254	17.8
65-69	12	1.03	67.7	9.8	102	8.7
70-74	21	3.24	72.6	7.4	139	21.5
75-79	12	1.94	77.5	5.8	63	10.3
80-84	11	2.93	82.4	4.5	46	12.2
85+	7	2.95	89.0	2.9	20	8.3
Total	105	0.46	69.1	10.1	885	3.8
Females						
45-49	1	0.05	47.7	30.5	20	1.0
50-54	3	0.23	52.6	25.5	53	4.1
55-59	8	0.48	57.7	19.9	120	7.1
60-64	8	0.46	62.6	16.8	106	6.1
65-69	16	1.11	67.6	13.8	180	12.5
70-74	23	2.45	72.6	10.1	201	21.4
75-79	15	1.48	77.6	8.1	107	10.6
80-84	12	1.83	82.6	6.4	70	10.6
85+	12	2.47	90.0	3.2	36	7.4
Total	98	0	73.0	11.1	894	3.7

Abbreviations: Av. Age at death =average age at death; Standard LE=Standard life expectancy; YLL= Years of life lost

Years of life lost (YLL) is an indicator which takes into account the age at which premature deaths occur by giving greater weight to deaths at younger age and lower weight to deaths at older age [22]. Disability-Adjusted Life Year (DALY) is another quantifier of the Burden of Disease from mortality and morbidity; DALYs are calculated as the sum of the YLL due to premature mortality in the population and the Years Lost due to Disability (YLD) for people living with a specific health condition or its consequences [25].

YLL is calculated by totalling the number of deaths at each age between 1-79.1 years for women and between 1-71.7 years for men, multiplied by the number of years of life remaining up to the age of 79.1 years for women and 71.7 for men, according to the Life Expectancy tables [19], added up to 1,779 years in men and women for all cases in this study. DALY was established with calculation template available on World Health Organization site [26].

Premature death YLL selects mainly men aged 60-64 and women aged 70-74 cut also affects active for society individuals aged 45-59 both gender, YLLs for them being 433, meaning 24.33% of all premature deaths, Table 4.

Total mortality accounted for 7.88 % of DALY, meaning 8.44% for men and 7.38% for women, table 5.

YLDs induced by all these three health conditions were 9,590 for men and 11,205 for women, total 20,795. DALYs, or burden of disease were 22,574 in men and women (10,475 in men and 12,098 in women). YLDs accounted for 92.119% of DALY for all inpatients. The burden in men, and the share of the YLD component in men reached 46.40% of total DALY. For women the YLD component reached 53.59% of total DALY, Table 6. YLD selects mainly inpatients aged 60-69, without regard to gender, total YLD=14,298.

Mortality alone does not give a real picture of the burden of disease, but DALY is capable to give an indication of the overall burden of it. One DALY represents the loss of the equivalent of one year of full health. Using DALYs, the burden of diseases that cause premature death but little disability (such as drowning or measles) can be compared to that of diseases that do not cause death but do cause disability

	Deaths	Deaths per 1,000	Av. Age at death	YLLs	YLL per 1,000
Males					
30-44	1	0.0	37.5	21	0.4
45-59	17	0.3	54.1	241	5.0
60-69	36	1.4	64.3	356	13.7
70-79	33	2.6	74.3	202	16.0
80+	18	2.9	85.0	66	10.7
Total	105	0.5	69.1	885	3.8
Females					
45-59	12	0.2	55.6	193	3.8
60-69	24	0.8	66.0	286	9.0
70-79	38	1.9	74.6	309	15.8
80+	24	2.1	86.3	106	9.3
Total	98	0.4	73.0	894	3.7

Abbreviations: Av. Age at death =average age at death; YLL= Years of life lost

Table 5
YLL IN STUDY AGE GROUPS FOR DM, PAD AND RELATED DERMATOLOGICAL CONDITIONS

	Incidence	Incidence per 1,000	Age at onset	Duration (years)	YLDs	YLD per per 1,000
Males						
0-4	30	0	2.5	0.0		0.0
5-14	45	0	10.0	0.0		0.0
15-29	145	0	22.5	0.0		0.0
30-44	354	0	37.5	0.0		0.0
45-59	1,497	0	52.5	0.0		0.0
60-69	1,668	2	65.0	10.0	7,205	277.5
70-79	840	10	75.0	5.0	1,950	154.2
80+	303	30	85.0	3.0	435	71.0
Total	4,882	21.2	60.0	4.5	9,590	41.7
Females						
0-4	46	0	2.5	0.0		0.0
5-14	55	0	10.0	0.0		0.0
15-29	110	0	22.5	0.0	0	0.0
30-44	184	0	37.5	0.0	0	0.0
45-59	1,508	0	52.5	0.0	3	0.1
60-69	1,642	3	65.0	10.0	7,093	223.7
70-79	1,430	15	75.0	5.0	3,320	169.8
80+	550	40	85.0	3.0	789	69.0
Total	5,525	22.7	63.3	4.6	11,205	45.9

Abbreviations: YLD= Years Lost due to Disability

Table 6
YEARS LOST DUE TO DISABILITY

Total DALYS = YLL+YLD						
	Males		***Females***		***Persons***	
Age	**DALYs**	**DALYs per1,000**	**DALYs**	**DALYs per1,000**	**DALYs**	**DALYs per1,000**
30-44	21	0.4			21	0.2
45-59	241	5.0	96	3.9	437	4.4
60-69	7,561	291.2	7,379	232.7	14,940	259.0
70-79	2,152	170.2	3,628	185.6	5,781	179.5
80+	500	81.7	895	78.3	1,395	79.5
Total	10,475	45.5	12,098	49.6	22,574	47.6

Abbreviations: DALY= Disability-Adjusted Life Year

Table 7
TOTAL DALYs FOR DM, PAD AND DERMATOLOGICAL CASES

Table 8
BURDEN OF NONTRAUMATIC AMPUTATIONS IN ARAD COUNTY, 2014-2018

	Deaths per 1,000	Av. Age at death	YLLs	YLL per 1,000	Age at onset	Duration (years)	YLDs	DALYs	DALYs per 1,000
Males									
30-44					37.5	0.0			
45-59	0.1	56.4	53	1.1	52.5	0.0		53	1.1
60-69	0.2	63.5	61	2.4	65.0	10.0	1,037	1,098	42.3
70-79	0.4	74.5	30	2.4	75.0	5.0	316	346	27.4
80+	0.7	84.1	15	2.5	85.0	3.0	73	88	14.5
Total	0.1	69.2	160	0.7	65.4	5.5	1,426	1,586	6.9
Females									
30-44					37.5	0.0	0		
45-59					52.5	0.0	0		
60-69	0.2	66.6	58	1.8	65.0	10.0	380	438	13.8
70-79	0.4	75.1	64	3.3	75.0	5.0	186	249	12.8
80+	0.4	84.4	20	1.8	85.0	3.0	55	75	6.6
Total	0.1	74.8	142	0.6	70.1	6.0	620	763	3.1

[27], (such as cataract causing blindness or amputation causing a devastating and life-changing experience).

Finally, amputations only, as disease burden, were responsible, in five years, of 2,348 DALYs for all persons, of which 1,586 were for men and 763 for women, with premature total deaths YYL of 302 and YDLs of 2,046, tabel 8, representing 10% of total DALYs, 9.83% of YLDs and 16.97% of YYLs.

Conclusions

DM-associated atherosclerosis can lead to complications in all major of vascular beds, including the coronary arteries, carotid vessels, and lower extremity arteries [28,-30]. Free radicals are implied in these mechanisms [31,32]. Antioxidant mechanisms are activated and antioxidant treatments try to limit the consequences [33]. Moreover, 20-30% of patients with PAD have DM, although this is likely underestimated by the asymptomatic nature of less severe PAD and the altered pain perception in diabetic patients due to peripheral neuropathy [34]. Revascularization attempts can often be difficult [35]. Here is how the perception of a problem can be changed by the angle of view from which we look [36-44]. This study emphasised the connection between them in terms of pathophysiological mechanisms and severity of complications, both having a huge impact as DALYs contributors, shortening life expectancy and dramatically decreasing the quality of life.

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