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Granulation/Tabletting of Highly Dosed Drugs Using Different Techniques: A Comparative Study

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Abstract

Paracetamol tablets and cimetidine tablets were prepared by single-step granulation/tabletting and by compression after high shear granulation. The addition of PVP (polyvinylpyrrolidone) was essential for single-step granulation/tabletting of formulation containing high concentrations of paracetamol or cimetidine. Paracetamol tablets without and with PVP obtained by single-step granulation/tabletting exhibited a significantly higher tensile strength, a significantly lower disintegration time, a lower friability and a faster dissolution compared to those prepared by compression after high shear granulation. Cimetidine tablets with PVP obtained by single-step granulation/tabletting exhibited a significantly lower tensile strength, a significantly lower disintegration time and a faster dissolution compared to those prepared by compression after high shear granulation. Single-step granulation/tabletting allowed to produce tablets containing up to 80% paracetamol or cimetidine with a dissolution profile complying with the USP requirements. For pure paracetamol or pure cimetidine the addition of crospovidone as a disintegrant was required to obtain a dissolution profile that complied with the pharmacopoeial requirements. Long term and accelerated stability studies of paracetamol tablets produced by single-step disintegration time was observed, it remained below 10 min. These results indicated that single-step granulation/tabletting could be an efficient technique for the production of highly dosed drugs such as paracetamol and cimetidine.

Keywords: Granulation/tableting; Dissolution; High dosage drugs; Paracetamol; Cimetidine.

1. Introduction

The pharmaceutical industry often faces difficulties in the processing of high dosed drugs because many active substances have poor compactability, poor disintegration or bad flow properties. Using conventional tabletting techniques, suitable excipients are required in order to obtain satisfactory tablets containing such drugs. However, for highly dosed drugs, the percentage of excipient that can be incorporated in the formulation is limited. For some highly dosed drugs an improved compactability could be obtained by changing the morphology of the drug crystals, but this requires strict process control and can result in the incorporation of impurities in the

drug crystals [1-4]. Recent experiments revealed that single-step granulation/tabletting of α-lactose monohydrate yielded stronger and faster disintegrating tablets compared with those produced by conventional compression [5, 6]. Further investigation of these tablets indicated that their high tensile strength was attributed to a different bonding mechanism, while the faster disintegration was explained by a higher porosity and a larger pore size. This technique might therefore be useful for processing highly dosed drugs with poor compaction or disintegration properties.

Paracetamol is a highly dosed, poorly water soluble drug possessing a poor compactability and yielding tablets exhibiting a high tendency to cap and laminate. Cimetidine is a highly dosed drug exhibiting good compactability, but poor flow and poor tablet disintegration properties. The aim of this study was to assess the suitability of single-step granulation/tabletting for the production of highly dosed paracetamol and cimetidine tablets and to compare the properties of these tablets with those obtained by compression after high shear granulation. The influence of storage on the stability of paracetamol tablets produced by single-step granulation/tabletting was assessed.

2. Materials and Methods

2.1. Materials

α-Lactose monohydrate, with an average particle size of 36 μm, (Pharmatose® 200M) was obtained from DMV (Veghel, The Netherlands), cimetidine, with an average particle size of 61 μm, was purchased from Roig Farma (Barcelona, Spain), paracetamol with an average particle size of 139 μm, was obtained from Mallinckrodt (Capitol Boulevard, NC, USA). Polyvinylpyrrolidone (PVP, Kollidon® K30) and crospovidone (PVP-CL, Kollidon® CL) were received from BASF (Ludwigshafen, Germany).

2.2. Single-step granulation/tabletting

Single-step granulation/tabletting was performed on a MP 19 TC 25 laboratory scale co-rotating twin screw extruder (APV Baker, Newcastle-under-Lyme, UK) having a length - to - diameter ratio of 25/1, equipped with a standard screw profile and a circular 9 mm die attached to the extruder outlet [5]. Processing parameters were set at 25°C barrel temperature, 250 rpm screw speed and 5.6 kg/h total input rate (powder and binding liquid). The water concentration was optimised for each formulation. When α-lactose monohydrate or crospovidone was

incorporated in the formulation it was preblended with the drug for 15 min at 60 rpm in a planetary mixer (Kenwood Major, Hampshire, UK). The granulation liquid (pure water or a 2.5% aqueous PVP solution) was pumped into the barrel by means of a peristaltic pump (Watson Marlow, Cornwall, UK). Immediately after extrusion, tablets (thickness: 4 mm) were manually cut using surgical blades [5]. The tablets were oven-dried at 25°C for 20 h. All water concentrations were calculated based on the wet mass, while PVP, cimetidine and paracetamol concentrations were calculated based on dry weight.

Single-step granulation/tabletting was characterised by monitoring the following process parameters: die pressure, power consumption and barrel temperature. If the power consumption exceeded 80% of its maximum or the die pressure was above 5 bar the process was stopped in order to avoid machine damage.

2.3. High shear granulation and tabletting

High shear granulation was performed in a Gral 10 (Machines Collette, Wommelgem, Belgium). When α-lactose monohydrate or crospovidone was incorporated in the formulation it was blended with the drug for 15 min at 60 rpm in a planetary mixer (Kenwood Major, Hampshire, UK). The granulation process was performed at 500 rpm impeller speed, 3000 rpm chopper speed, a total load of 0.16 $kg.l^{-1}$ and 10% water concentration. After a 2 min mixing period of the powder, the required amount of granulation liquid (water or a 2.5% aqueous PVP solution) was continuously added over a period of 10 min using a peristaltic pump (Watson Marlow, Cornwall, UK). Wet massing was continued for 2 min following complete liquid addition. Granules were oven dried at 25°C for 20 h.

The granules ($F_{250\text{-}710\ \mu m}$) were blended with 0.5% (w/w) magnesium stearate (<90 µm) (BUFA, Brussels, Belgium) in a Turbula mixer (W.A. Bachofen, Basel, Switzerland) for 1 min. Tablets (250 mg) were prepared using an eccentric compression machine (Korsch EKO, Berlin, Germany) equipped with a flat faced double punch of 9 mm at a compression force of 10 kN per tablet. Similarly, tablets were prepared (without magnesium stearate) with die lubrication using magnesium stearate solution (1%, w/v) in ethanol.

2.4. Tablet evaluation

Immediately after production tablets were stored at 25°C and 60% RH for 24 h prior to evaluation.

2.4.1. Tablet friability

The tablet friability was determined using a friabilator (Pharma Test, Hainburg, Germany) at a speed of 25 rpm for 4 min. The percentage weight loss was expressed as the tablet friability.

2.4.2. Tablet porosity

The tablet porosity ε was determined (n=3) using He-pycnometry (Micromeritics, Norcross, GA, US) by the following equation ε = (bulk volume – skeletal volume)/ bulk volume × 100.

2.4.3. Tablet tensile strength

The hardness, thickness and diameter of the tablets (n=6) was determined (Pharma Test, Hainburg, Germany). The tablet tensile strength T was calculated using the equation described by Fell and Newton [7],

$$T = 2F/(\pi.d.t)$$

where F, d and t denote the diametral crushing force, the tablet diameter and the tablet thickness, respectively.

2.4.4. Disintegration time

The disintegration time was determined (n=6) using the apparatus described in Eur. Ph. III (Pharma-Test, Hainburg, Germany). Tests were performed in distilled water at 37°C using disks.

2.4.5. Dissolution test

Dissolution tests of paracetamol tablets were performed in 900 ml phosphate buffer (pH 5.8) using the paddle method (Vankel, Cary, NC, US). The dissolution medium was maintained at 37 ± 0.5°C, while the rotation speed was set at 50 rpm (USP XXIV). Samples (5 ml) were withdrawn after 5, 10, 15, 20, 25 and 30 min and the paracetamol concentration was determined spectrophotometrically at 243 nm (Lambda 12 Perkin Elmer, Norwalk, US). Similarly dissolution tests were performed on cimetidine tablets in 900 ml water using the basket method (Vankel, Cary, NC, US). The dissolution medium was maintained at 37 ± 0.5°C, while the rotation speed was set at 100 rpm (USP XXIV). Samples (5 ml) were withdrawn after 3, 6, 9, 12 and 15 min and the cimetidine concentration was determined spectrophotometrically at 218 nm (Lambda 12 Perkin Elmer, Norwalk, US).

2.5. Tablet stability

Tablets without PVP containing 20% paracetamol and with 2.5% PVP containing 80% paracetamol, prepared by single-step granulation/tabletting,

were stored at 60% RH and 25°C and at 75% RH and 40°C for one year. Tablets were evaluated for tensile strength, friability, disintegration and dissolution after 1, 90, 270 and 360 days.

2.6. Statistical analysis

Statistical analysis was performed using the computer program SPSS version 11.0. Statistically significant differences between the tensile strength and disintegration time of tablets produced by single-step granulation/tabletting and by compression after high shear granulation as well as the influence of storage time and storage conditions on tablet tensile strength and disintegration time were determined using a one-way ANOVA. For further comparison of the influence of production technique, storage time and condition on the tablet properties a multiple comparison among pairs of means performed using Scheffé test with $P < 0.05$ as a significance level was used. The data were tested for normal distribution with a Kolmogorov-Smirnov test. The homogeneity of variances was tested with the Levene's test.

3. Results and Discussion

3.1. Process characterization

Table 1 shows the process parameters obtained during preparation of paracetamol and cimetidine tablets by single-step granulation/tabletting.

Table (1): Process parameters during single-step granulation/tabletting of formulations containing paracetamol and cimetidine.

Formulation				Process parameters		
Drug (%)	**PVP (%)**	**PVP-CL (%)**	**Water (%)**	**Die Pressure (bar)**	**Barrel temp (°C)**	**Power consumption (%)**
Paracetamol						
20	0	0	11.5	1	33	22
40	0	0	11.5		Processing not feasible	
100	0	0	11.5		Processing not feasible	
20	2.5	0	9.5	0	33	19
40	2.5	0	9.5	1	33	22
60	2.5	0	9.5	1	31	19
80	2.5	0	10.5	3	30	25
97.5	2.5	0	10.5	3	35	27
92.5	2.5	5	10.5	0	38	30
Cimetidine						
20	0	0	17.5		Processing not feasible	
100	0	0	11.5-25.0		Processing not feasible	
80	2.5	0	14.5	0	36	25
97.5	2.5	0	14.5	0	36	34
92.5	2.5	5	14.5	0	33	31

Paracetamol processing without PVP was only possible at a drug load of 20%. Processing of formulations containing 40% paracetamol or more created too much friction inside the extrusion barrel and process blocking within the first few minutes of the process (even at higher water concentrations).

Cimetidine processing without PVP was not possible even at a drug load of 20%. The fact that single-step granulation/tabletting of pure cimetidine and paracetamol without PVP was not possible at the set parameters was probably due to their physical properties. Both drugs have lower (1 in 70 for paracetamol and 1 in 88 for cimetidine) water solubility than α-lactose monohydrate 200M (1 in 5), which was easily processed at the same settings. A formulation containing 20% paracetamol could be processed whereas, single-step granulation/tabletting of a mixture containing 20% cimetidine was not feasible can be attributed to their different particle size.

This is in agreement with previous experiments showing that the feasibility of the single-step granulation/tabletting depends on the particle size [8]. Addition of 2.5% PVP improved processing and allowed single-step granulation/tabletting of formulations containing up to 97.5% of both drugs investigated. Smooth surfaced extrudates were obtained at all concentrations except for 97.5%, which had minor surface roughness. This roughness disappeared on addition of 5% crospovidone. When processing 97.5% cimetidine, a higher water concentration was required than for the 97.5% paracetamol formulation. This difference could again be attributed to the differences in particle size.

Table (2): The influence of storage on the properties of tablets (without PVP) containing 20% paracetamol and tablets (with 2.5% PVP) containing 80% paracetamol produced by single-step granulation/tabletting.

			60% RH-25 °C			75% RH-40 °C		
Time (d)	**PCM** (%)	**PVP** (%)	**Tensile strength** (MPa)	**Friability** (%)	**Dis[a]** (s)	**Tensile strength** (MPa)	**Friability** (%)	**Dis[a]** (s)
1	20	0	0.84 (0.19)	0.44	32 (6)	0.88 (0.12)	0.84	36 (8)
60			0.76 (0.11)	0.68	39 (5)	1.07 (0.14)	0.66	72 (29)
180			0.81 (0.19)	0.83	33 (17)	0.98 (0.25)	0.68	86 (19)[b]
360			0.88 (0.20)	0.88	70 (15)[b]	0.95 (0.18)	0.68	119 (20)[b]
1	80	2.5	1.39 (0.21)	0.99	112 (23)	1.24 (0.11)	1.02	91 (18)
60			1.40 (0.27)	0.95	109 (23)	1.37 (0.32)	0.75	121 (23)
180			1.43 (0.21)	0.72	130 (31)	1.42 (0.22)	0.97	173 (25)
360			1.50 (0.47)	0.97	201 (31)[b]	1.27 (0.37)	0.83	210 (48)[b]

[a] Disintegration. [b] Significantly different from the other values in the same group. Standard deviations are given between parentheses (PCM, paracetamol; Dis, disintegration)

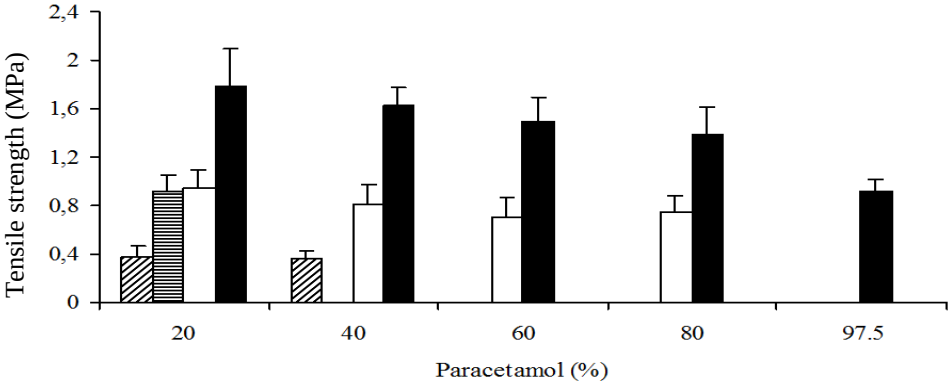

Figure (1. a.) Tensile strength of paracetamol tablets produced with high shear granulation without PVP ( ) and with 2.5% PVP ( ) and by single-step granulation/tabletting without ( ) and with 2.5% PVP ( ).

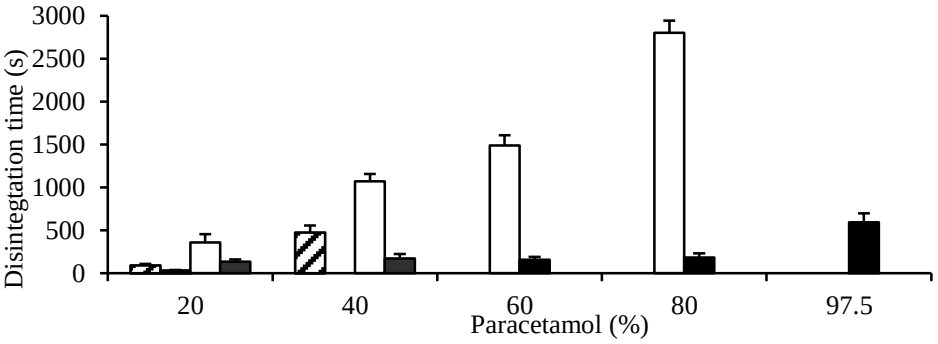

Figure (1. b.) Disintegration of paracetamol tablets produced by high shear granulation without PVP ( ) and with 2.5% PVP ( ) and by single-step granulation/tabletting without PVP ( ) and with 2.5% PVP ( ).

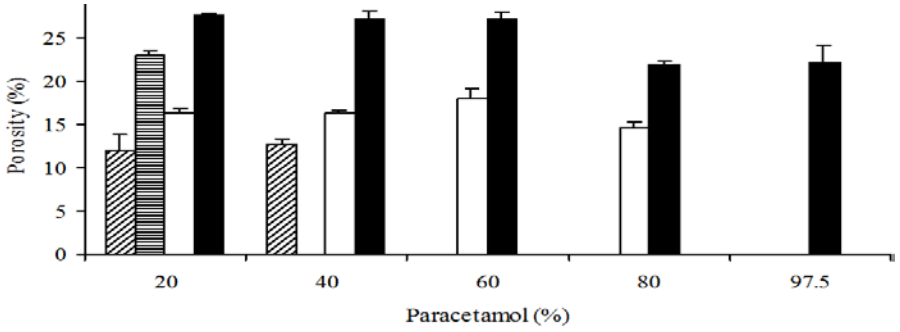

Figure (1. c.) Porosity of paracetamol tablets produced with high shear granulation without ( ) and with 2.5% PVP ( ) and by single-step granulation/tabletting without ( ) and with 2.5% PVP ( ).

3.2. Tablet properties

Figure (1a) shows the tensile strength of paracetamol tablets produced by single-step granulation/tabletting and by compression after high shear granulation.

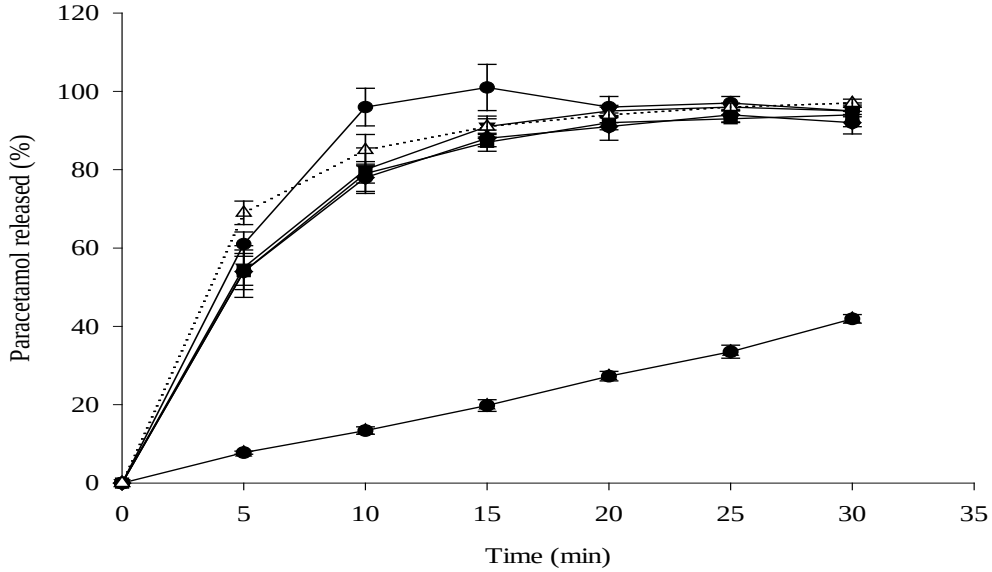

Figure (2 a.) Dissolution profiles of tablets with 2.5% PVP containing 20% (λ), 40% (5), 60% (ν), 80% (♦), 97.5% (σ) paracetamol and 92.5% (6) paracetamol with 5% crospovidone prepared by single-step granulation/tabletting process.

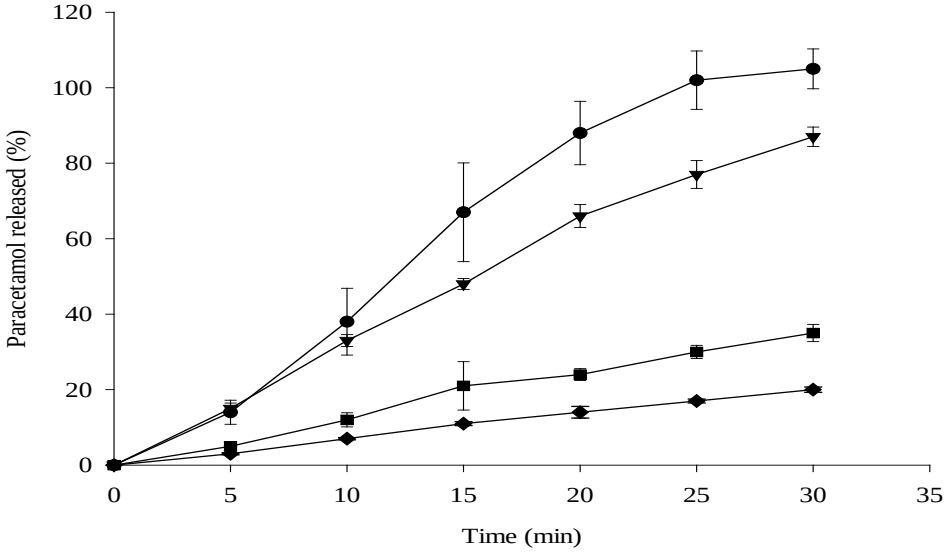

Figure (2 b.) Dissolution profiles of tablets containing 20% (λ), 40% (5), 60% (ν) and 80% (♦) paracetamol prepared by compression after high shear granulation.

Tablets without PVP containing 20% paracetamol prepared by single-step granulation/tablet-ting showed an average tensile strength of 0.92 MPa. Although, high shear granulation of formulations without PVP containing more than 20% paracetamol was feasible, no acceptable tablets could be prepared from those granules. For the formulation containing up to 40% paracetamol the tablet tensile strength was below 0.60 MPa for tablets prepared with blending with magnesium stearate and with die lubrication. However, no tablets were obtained from granules containing 60% paracetamol due to the tendency for capping and lamination. These data are in agreement with Becker *et al.* [9] who reported that paracetamol tablets produced without binder had a poor strength.

Addition of 2.5% PVP to paracetamol formulations resulted in a significant increase in tensile strength for both production techniques. This resulted in hard tablets up to a paracetamol load of 97.5% when prepared by single-step granulation/tabletting. On the contrary, the formulation containing 97.5% paracetamol still showed poor compactability after high shear granulation for both formulation prepared by blending with magnesium stearate and die lubrication. Becker *et al.* [9] and Symecko *et al.* [10] reported similar results and stated that at least 5% binder (e.g. PVP) is required to obtain paracetamol tablets with an adequate tensile strength. The results of tablet tensile strength of both granulation techniques indicated that single step granulation/tabletting resulted in improved tensile strength mainly due to the different bonding mechanism, which come in agreement with data reported by Keleb *et al* [8] for different grades of lactose and with data reported by Djuric and Kleinebudde [9].

Comparison of the tensile strength obtained by both techniques revealed a significantly higher tensile strength for all paracetamol tablets produced by single-step granulation/tableting. This higher tensile strength can be explained by the different bonding mechanism involved in both techniques [11]. By conventional compression, bonding occurs mainly through intermolecular bonds. Paracetamol exhibits poor compactability due to the weak intermolecular bonds established during compression. In single-step granulation/tabletting bonding occurs mainly through solid bridges formed during drying by resolidification of the material dissolved during the process [5, 12] and these solid bridges are much stronger than intermolecular forces [12]. Evaluation of the effect of paracetamol concentration on tensile strength revealed that only an increase of the paracetamol concentration to 97.5% resulted in a significant decrease of

tensile strength. For all formulations the friability of tablets produced by single-step granulation/tabletting ranged between 0.68 and 0.91%, whereas tablet friability was above 1% for all tablets produced by high shear granulation and compression, regardless of the lubrication method employed. The addition of PVP resulted in a considerable reduction of the tablet friability, but did not reduce the friability below 1%. Figure (1b) shows the disintegration time of paracetamol tablets produced by single-step granulation/tabletting and by compression after high shear granulation. For all paracetamol tablets produced by single-step granulation/tabletting the disintegration time remained below 10 min and was significantly lower than that of tablets made by high shear granulation and compression. This faster disintegration can be attributed to the higher tablet porosity as shown in Figure (1c). For both production techniques, tablets containing 2.5% PVP showed a significantly higher disintegration time than those without PVP.

For tablets processed by single-step granulation/tabletting increasing the paracetamol concentration resulted in a significantly higher disintegration time only when the drug load was increased to 97.5%, while for tablets produced by high shear granulation and compression the disintegration time increased progressively in function of drug concentration. For tablets containing more than 20% paracetamol prepared by high shear granulation and compression the addition of PVP resulted in a disintegration time above 15 min.

At increasing paracetamol concentration the fraction of α-lactose monohydrate, (having a 20-fold higher aqueous solubility) decreased and thus the overall solubility of the formulation decreased. This decrease in solubility clearly affected the disintegration time of tablets with low porosity Figure (1c) obtained by high shear granulation and compression. The fact that this did not affect the disintegration time of tablets produced by single-step granulation/tabletting indicated that the higher porosity allowed to compensate for the differences in solubility. Results of the dissolution testing of paracetamol tablets are shown in Figure (2a) and (b) Evaluation of the dissolution profiles revealed that tablets prepared by single-step granulation/tabletting exhibited faster dissolution. As for the disintegration, this faster dissolution can be explained by the higher porosity of tablets produced by single-step granulation/tabletting. Tablets produced by single-step granulation/tabletting complied with the USP requirements up to a drug load of 80%, while tablets produced by compression after high shear granulation did only comply up to a drug load of 40%.

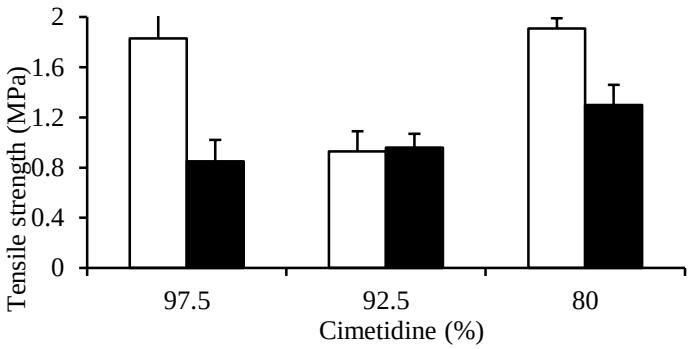

Figure (3a.) Tensile strength of cimetidine tablets containing 2.5% PVP produced by high shear granulation (□) and by single-step granulation/tabletting (■)

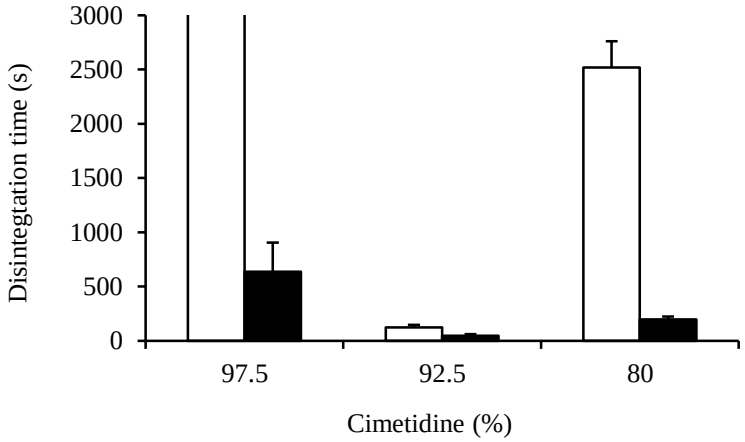

Figure (3b.) Disintegration of cimetidine tablets containing 2.5% PVP produced by high shear granulation (□) and by single-step granulation/tabletting (■).

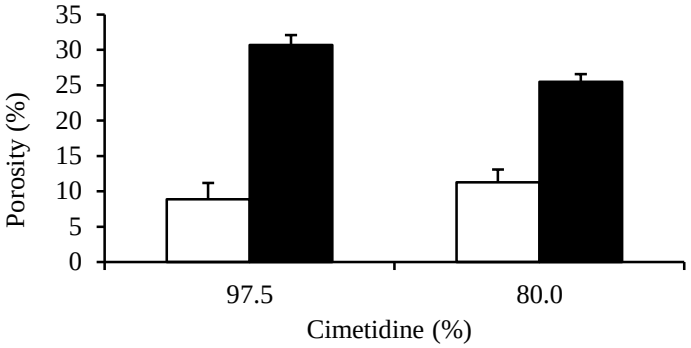

Figure (3c.) Porosity of cimetidine tablets containing 2.5% PVP produced by high shear granulation (□) and by single-step granulation/tabletting (■).

Evaluation of the different tablet properties show that by compression, no acceptable tablets could be obtained without PVP and acceptable tablets were only obtained with 2.5% PVP up to 40% paracetamol. This clearly showed that for formulations with poor compaction properties single-step granulation/tabletting allowed to manufacture quality tablets whereas conventional high shear granulation and tabletting failed. The potential of the single-step granulation/tabletting process for producing tablets of formulations with poor disintegration and flow properties was assessed using cimetidine as a model drug.

Figure 3a, b and c show the tensile strength, the disintegration time and the porosity of cimetidine tablets produced by single-step granulation/tabletting and by compression after high shear granulation. Tablets containing 97.5% cimetidine and 2.5% PVP produced by single-step granulation/tab-letting showed a significantly lower tensile strength (0.85 MPa), a significantly lower disintegration time (< 15 min), lower friability of 0.93% and faster dissolution than those produced by high shear granulation and compression (with blending with magnesium stearate). Similarly single step granulation/tabletting showed a significantly lower tensile strength and lower disintegration time than tablets produced after high shear granulation and compression with die lubrication (data not shown). These results indicated that the improved disintegration for tablets produced by single step granulation/tabletting is mainly due to the higher porosity.

Although single-step granulation/tabletting resulted in an improved disintegration time of cimetidine tablets mainly due to the higher porosity Figure (3c), incorporation of 17.5% α-lactose monohydrate 200M in the cimetidine formulation with PVP yielded tablets with a significantly lower disintegration time (198 s), a significantly lower tensile strength (1.30 MPa) and faster dissolution than those produced by high shear granulation and compression. The dissolution profile of these cimetidine tablets with 17.5% α-lactose monohydrate complied with the pharmacopoeial requirements Figure (4). In another attempt to improve the disintegration and dissolution of pure cimetidine tablets, 5% crospovidone was added. This resulted in a disintegration time below 3 min Figure (3b). The tablets produced by both techniques complied with the USP requirements (not less than 75% of the labelled amount dissolved in 15 min) Figure (4).

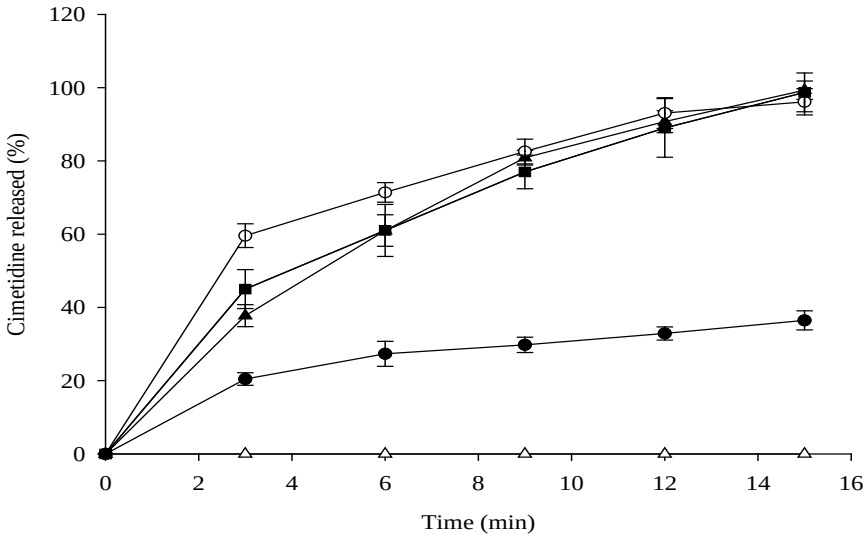

Figure (4): Dissolution profiles of cimetidine tablets with 2.5% PVP produced by single-step granulation/tabletting without (λ), with 5% crospovidone (O), with 17.5% α-lactose monohydrate 200M (ν) and by compression after high shear granulation without (6) and with 5% crospovidone (σ).

3.3. Stability of paracetamol tablets produced by single-step Granulation/Tabletting

As it is known that tablet properties can be affected by storage conditions, the stability of tablets produced by single-step granulation/tabletting was evaluated during one year storage at 60% RH-25°C and 75% RH-40°C. This stability study was performed on tablets without PVP containing 20% paracetamol and with 2.5% PVP containing 80% paracetamol. Table 2 shows the influence of storage on the properties of those tablets. It is obvious that storage had no significant influence on the tensile strength, but resulted in a significant increase in the disintegration time for both formulations studied.

However, the disintegration time remained below 5 min. The increase in disintegration time could be explained by a minor decrease in the tablet porosity. Additional porosity measurements after one year revealed that storage of tablets at 75% RH–40°C resulted in a reduction of the porosity from 23 and 22% to 18.7 (± 0.85) and 19.3% (± 0.73) for formulations containing 20 and 80% paraceatmol, respectively. However, no influence on the tablet porosity was observed for tablets stored at 60% RH–25°C. For both formulations no change in dissolution profile was observed over a period of one year as shown in Figure (5).

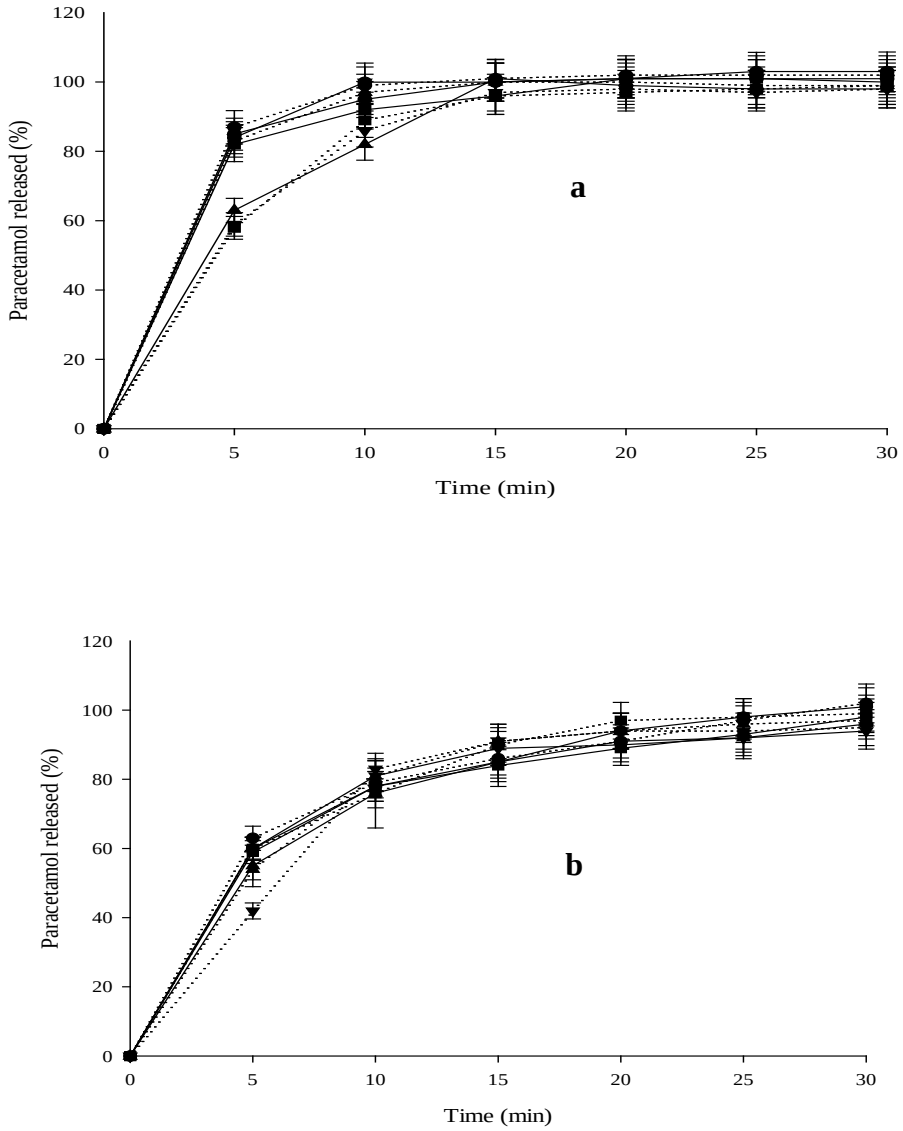

Figure (5): Dissolution profiles of (a) tablets containing 20% paracetamol without PVP and (b) tablets with 2.5% PVP containing 80% paracetamol prepared by single-step granulation/tabletting after storage for 1, (λ), 90 (ν), 270 (σ) 360 days (5) at 60% RH–25°C (___) and at 75% RH–40°C (-----), respectively.

It is clear that paracetamol tablets produced by single step-granulation/tabletting were not influenced by the storage conditions. After a storage period of one year, these tablets had a high tensile strength, fast disintegration time and a dissolution profile that complied with the pharmacopoeial requirements. Whereas, paracetamol tablets produced by

compression after wet granulation exhibited a significant increase in the tablet tensile strength and disintegration time and a slower dissolution after storage, even though they contained disintegrant [12-14].

4. Conclusion

From these experiments it can be concluded that single-step granulation/tabletting allowed to produce acceptable tablets at a higher drug level than conventional high shear granulation and compression. Single-step granulation/tabletting allows to produce paracetamol and cimetidine tablets with acceptable tensile strength, fast disintegration time and dissolution profile that complied with the pharmacopoeial requirements up to a drug load of 80%. However, addition of PVP and incorporation of 5% crospovidone allowed to produce tablets containing 92.5% paracetamol or cimetidine that complied with the pharmacopoeial requirements.

The paracetamol tablets and the cimetidine tablets produced by single-step granulation/tabletting exhibited a higher tensile strength and faster disintegration compared with tablets produced by compression after high shear granulation. Paracetamol tablets produced by single-step granulation/tabletting were not influenced by the storage conditions investigated over a period of one year.

It can be concluded that single-step granulation/tabletting is an efficient tool for the preparation of tablets of highly dosed drugs and can significantly improve their tablet properties.

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Prevalence of Bacterial Hemolytic Strains and Their Antimicrobial Resistance Pattern in Relation to Antibiotic Misuse by Misurata Dental Patients

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Abstract

Fifty gum infected patients were swabbed for oral cavity to report the prevalence of heamolytic and non-heamolytic bacterial strains and their drug resistance condition against commonly used antibiotics. Obtained results revealed the predominance of heamolytic strains than non-heamolytic ones. In addition, both types showed high antimicrobial resistance patterns against most tested antibiotics. Positive correlations were observed between incidence of drug resistances and many unhealthy attributes like smoking, infrequent teeth brushings and mainly; uncontrolled use of antibiotics. Noteworthy was also that antimicrobial resistance was noticed mainly against amoxicillin/clavulonic acid, cefoxitin and trimethoprim/sulphamethoxazole. This study highlighted the hazard of misuse of antibiotics and the necessity of both controlling and awareness programs among both physicians and community.

Keywords: Antibiotic misuse; Antimicrobial resistance; Dental problems.

1. Introduction

A healthy gum is one that is free of pain and infection, with pink gum tissue, do not bleed on brushing and the mouth should be moist, with no evidence of tooth decay [1]. Gum disease is typically progressed slowly and painlessly, so it can easily reach an advanced state before patient is aware of its presence. Protection from gum diseases could be done through learning more about gum disease and taking action to ensure better overall health [2].

Gum disease or "clinically termed periodontal disease" involves destruction of tooth and supporting tissue (gum and bone),usually occur in a cyclic manner, with bursts of destruction and periods of inactivity [3]. The gum diseases or gum infections are responsible for 70 percent of all tooth diseases and are the principal cause of tooth extraction [4-7]. Periodontal disease can be defined as multifactorial infectious disease, being characterized by symptoms and clinical signs that may include inflammation (visible or invisible). Varying degrees of spontaneous gingival bleeding or bleeding on probing, pocket formation related to loss of attachment of

alveolar bone, and tooth mobility which may lead to tooth loss are associated with gum diseases [8].

Bacteria can produce toxins, which irritate the gum and cause infections [9]. In the early stage of gum disease, the gum can become red, swollen, and bleed easily, as it is not normal for gum to bleed [2]. Earlier intervention can minimize the damage to gum and teeth and positively impact overall health [10].

Many bacteria, fungi and viruses are associated with gum infections, although the major cause is bacteria, which infect gum, ligaments and supportive bones. Oral bacteria usually live in a film called plaque [11]. Plaque is a thick and sticky film of bacteria that's build up on the teeth, which could be hardened to become calculus, known also as tartar [12].

Plaque is spreading its growth on the teeth and down into the crevice on the gum and tooth, when the underlying bone is lost.This crevice is called the pockets. In very large amounts, plaque can be seen as a fuzzy unclean coating on the teeth.Bacteria of plaque may secrete toxin that damage the gums and underlying bone [13].

In dentistry, the routine use of antibiotics before or after extractions or endodontics has not been shown to be effective [14,15]. Therefore, routine prescribing for every extraction or endodontic procedure must be discouraged in order to fight the problem of antimicrobial resistance.

This study was conducted aiming many objectives. Initially, to detect microbial resistance among bacterial strains isolated from gum infection patients at Misurata's dental clinics. Secondly, to conclude the effect of different unhygienic and unhealthy habits on incidence of gum infections.

2. Materials and Methods

2.1. Patient Survey

A standardized survey designed to report many parameters. This survey provides a standardized methodology that allows objective and meaningful comparisons.The survey is composed of 9 questions. Question were designed to provide answer on different related topics to antibiotic resistance of oral and gum microbial infection. In addition, this survey was aimed to explore different habits that may affect gum health.

2.2. Collection of samples

Fifty samples of sub-gingival swab were collected during the period from 14 December 2013 to 18 March 2014, from"Misurata dental clinics" with the assistance of dentists and under aseptic conditions.The swab samples were sent to the laboratory as quickly as possible within 2 hours of collection.

2.3. Preparation of sample

Each swab sample was inoculated separately into nutrient broth and incubated at 37 ℃ for 24hr to enhance the growth of microorganisms.

2.4. Bacteriological examination of samples

2.4.1. Isolation of bacteria from sub-gingival swap sample

A loopful from incubated swab soaking nutrient broth were streaked on blood agar, chocolate agar and MacConkey agar and incubated at 37^{o}C for 24hr according to the method recommended by Arura and Arora [16].

2.4.2. Cultural characteristics

Cultural characteristics of isolated bacterial colonies after culturing them on different media such as size, shape and color were recorded.

2.4.3. Purification and Identification of isolated bacteria

Different colonies were picked up and streaked on slope agar, then incubated at 37^{o}C for 24hr for further purification and identification.

2.4.4. Microscopical examination

Gram stained film from bacterial growth was observed under oil immersion lens of a light microscope.The shapes of bacteria cell, stain reaction and arrangement were recorded.

2.4.5. Antimicrobial susceptibility testing

The susceptibility of bacterial isolates to antimicrobial agents was determined by using the disc diffusion method on nutrient agar according to the method recommended by Arura and Arora [16]. The bacterial isolates were evenly streaked on Muller hinton agar plates, then the antimicrobial discs were distributed on the plate and incubated for 18-24hr. About 5-6 discs were equally distributed on each cultured plate. Afterwards, the diameter of halo zone of non-growth around each disc was measured and the results were recorded as sensitive (+), relatively resistant (+/-), and resistant (-) and compared to Cappuccino and Sherman [17] Table (1).

Eleven antimicrobials were chosen to represent the mostly prescribed antimicrobials among dental clinics patients. The antimicrobial agents used in this study included: Tetracycline (30 µg), Amoxicillin/clavulanic acid (20/10µg), Erytromycin (30 µg), Chloramphenicol(30 µg), Nalidixic acid (30 µg), Ciprofloxacin (5µg), Nitrofurantoin (50 µg), Cefoxitin (30µg), Imipenem (10µg), Ceftriaxon (30µg) and Trimethoprim/sulphamethoxazole (1.25/23.75 µg).

Table (1): Interpretation chart of zone size in Kirby-Bauer disc diffusion method

Antimicrobial agent	Diameter of zone of inhibition (mm)		
	Resistant	Moderately susceptible	Susceptible
Erythromycin	≤13	14_17	≥18
Tetracycline	≤14	15_20	≥21
chloramphenicol	≤12	13_17	≥18
Nalidixic acid	≤13	14_18	≥19
Nitrofurantoin	≤14	15_17	≥18
Trimethoprim \ Sulphamethoxazole	≤10	11_15	≥16
Ciprofloxacin	≤15	16_20	≥21

3. Results and Discussion

This study was designed to isolate bacterial strains predominated in patients with periodontitis, to explore the antibiotic susceptibility to different antibiotics commonly prescribed as treatment or prophylaxis, and to study different factors affecting both prevalence of periodontal diseases and microbial resistance among them via a detailed questioner.

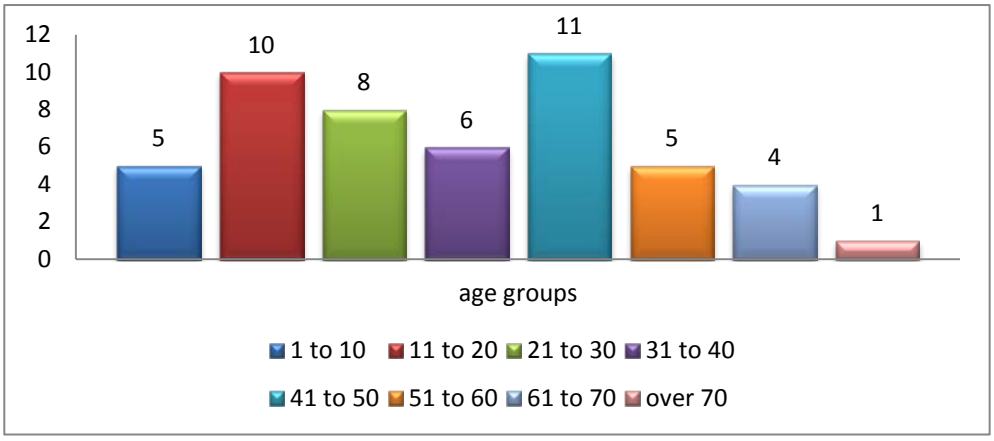

Figure (1): Groups of age of patient included in the study.

Regarding age of patients, Figure (1) presented a view for all the age of examined patients, and it indicated that the gum infection was most prevailed at age from 41-50 years. Very small population in the age group of 70 years was found to have a healthy periodontium. Although, periodontitis is also reported in different ages.

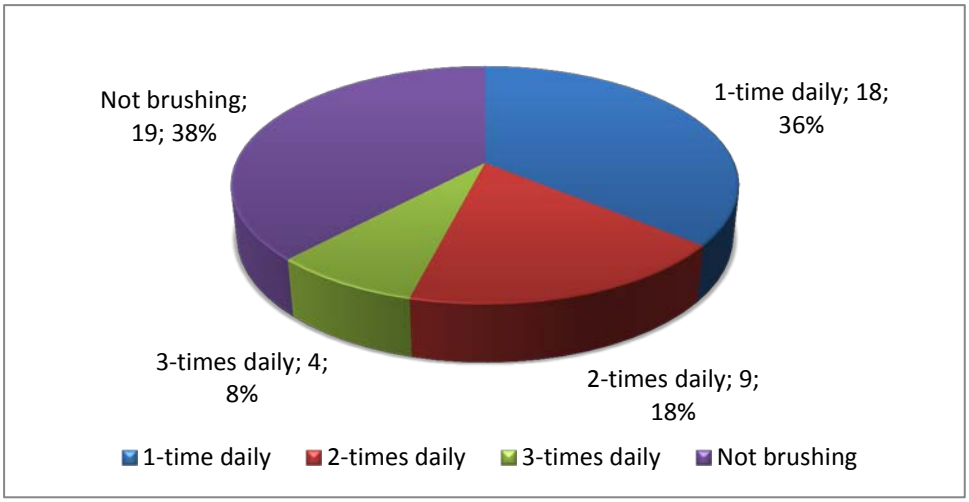

Figure (2): Distribution of patients according to frequency of daily teeth brushing.

Important factor affecting the incidence of periodontal diseases is the habit of proper cleaning of oral cavity via teeth brushing. Figure (2) presented that the majority of examined patients (38%) were not practicing brushing with a toothpaste, which represent a risky percentage when compared to previous study conducted on Libyan patients [37]. In addition, only 8 % of examined patients were practicing teeth brushing regularly of 3-times daily.It is very clear that the frequency of tooth brushing was significantly associated with the incidence of gum infections. While regarding the most important factor affecting the microbial resistance of antibiotics, the uncontrolled use of antibiotics was found to be of high impact.

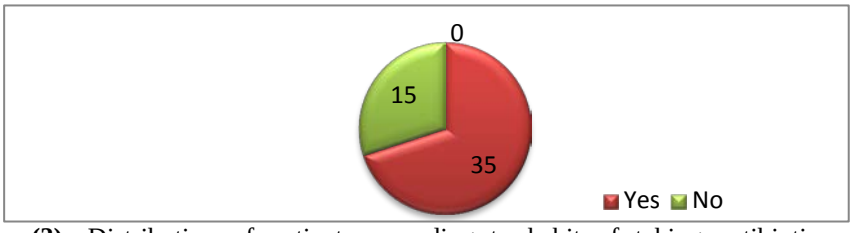

Figure (3): Distribution of patients according to habit of taking antibiotic without physician prescription.

Figure (3) showed that the majority of examined patients were taking antibiotics without physician's prescription. In line with previous study Peeran *et al* [18]**,** the severity of infection, Figure (4) showed that 40 % of examined patients suffered from gum bleeding. Different organisms were isolated from gum infections from included Misurata dental clinics' patients. Out of fifty swabs, which were collected from those patients, the number of isolated hemolytic bacterial strains was 29, which indicated the prevalence of hemolytic bacteria than non-hemolytic bacteria from those patients Figure (5).

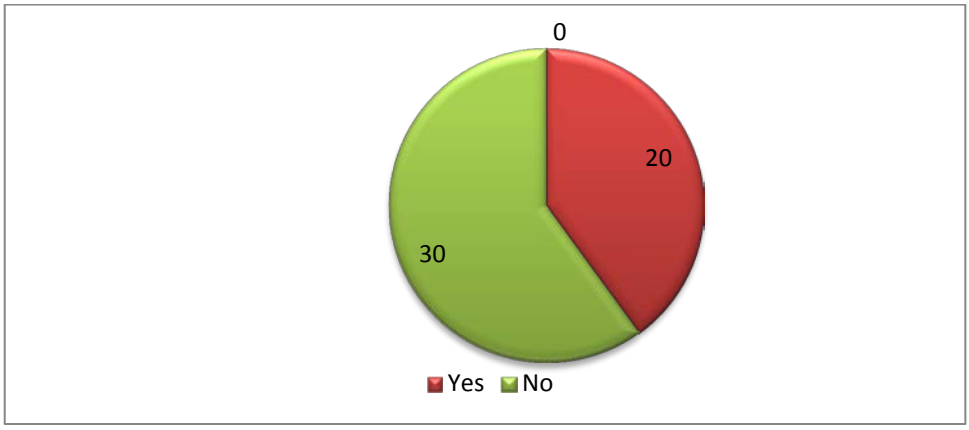

Figure (4): Distribution of patients according to history of gum bleeding.

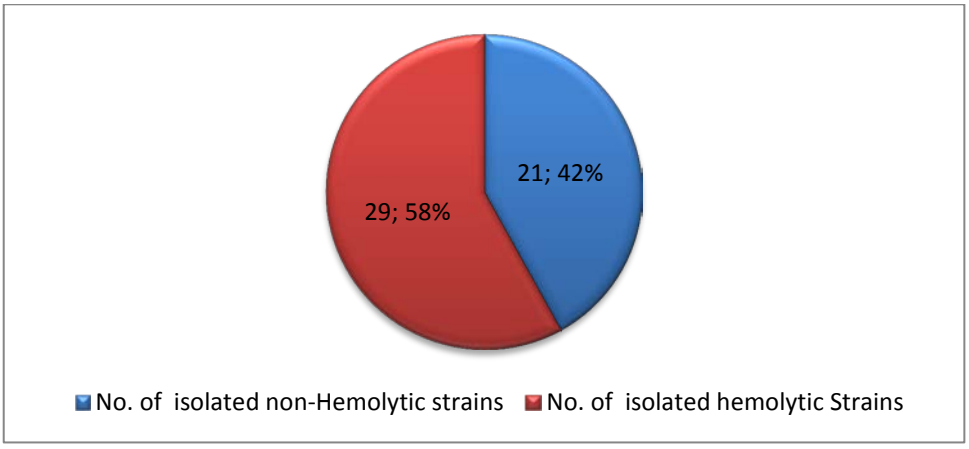

Figure (5): Prevalence of hemolytic and non-hemolytic strains isolated from gum infection Patients.

There are many international prevalence studies regarding isolation of microbial causative agents of gum infections, although, as far as our knowledge, only one literature regarding the gum infection status of the

population in Misurata by Khemaleelakul *et al*. [19], in which they reported nearly similar findings to our study except that the likelihood of the prevalence of periodontal disease was much less during adulthood. In order to determine the antimicrobial sensitivity pattern of isolated organisms, eleven antimicrobial agents were chosen in this study to compare the sensitivity of these isolated organisms. The selection of these antimicrobial agents was done based on the most comonly prescribed antimicrobial among Misurata Dental clinics.

Organisms varied in their response to these antimicrobial agents according to their types and/or strains differences. As for the antibiotic susceptibility of hemolytic isolated strains, the highest rate of resistance was found against Amoxicillin/Clavulanic acid and Trimethoprim/ Sulphamethoxazole, in which nearly 48 and 58 % of isolated heamolytic strains were resistant to these antimicrobial agents, respectively (Table 2). Imipenem and ciprofloxacin were found to have the greatest antimicrobial effect against heamolytic strains.

Table (2): Antibiotic resistance pattern of hemolytic isolated strains.

Antibiotics	**Percentage of resistant Strains**	**Percentage of intermediate Strains**	**Percentage of susceptible Strains**
Amoxicillin/Clavulanic acid	48.3	34.5	17.2
Trimethoprim/ Sulphamethoxazole	58.6	27.6	13.8
Cefoxitin	51.7	17.3	31.0
Erythromycin	37.9	44.8	17.3
Tetracycline	44.0	27.0	29.0
Imipenem	41.3	7.0	51.7
Ceftriaxone	44.8	20.7	34.5
Ciprofloxacin	38.0	10.0	52.0
Nalidixic Acid	69.0	20.7	10.3
Nitrofurantoin	34.5	34.5	31.0
Chloramphenicol	31.0	24.1	44.9

While regarding the antibiotic susceptibility of non-hemolytic strains, Amoxicillin/Clavulanic acid was found to be the most antimicrobial agent to which isolated organisms have developed a resistance against Table (3).

Table (3): Antibiotic resistance pattern of non-hemolytic isolated strains.

Antibiotics	Percentage of resistant Strains	Percentage of intermediate Strains	Percentage of susceptible Strains
Amoxicillin/Clavulanic acid	66.7	23.8	9.5
Trimethoprim/ Sulphamethoxazole	33.3	52.4	14.3
Cefoxitin	42.8	28.6	28.6
Erythromycin	52.4	33.3	14.3
Tetracycline	30.6	43.6	25.8
Imipenem	24.0	19.0	57.0
Ceftriaxone	33.0	48.8	19.0
Ciprofloxacin	24.0	24.0	52.0
Nalidixic Acid	33.3	52.4	14.3
Nitrofurantoin	57.2	33.3	9.5
Chloramphenicol	19.1	47.6	33.3

Similarly, to heamolytic strains, imipenem and ciprofloxacin were found to have the greatest antimicrobial effect against non-heamolytic strains. While in another comparison between heamolytic and non heamolytic strains Tables (4), (5) and (6) imipenem and ciprofloxacin were found to be the highly effective antimicrobial agents against both types of isolated strains (highly sensitive and less resistant strains). At the contrary, Nalidixic Acid was found to be the most non effective antimicrobial against heamolytic strains, and Amoxicillin/Clavulanic acid against non-heamolytic strains.

Table (4): Comparison of antibiotic significance among isolated susceptible hemolytic and non-hemolytic strains.

Antibiotics	No. of hemolytic Susceptible strains	No. of non-hemolytic Susceptible strains
Amoxicillin/Clavulanic acid	5	2
Trimethoprim/ Sulphamethoxazole	4	3
Cefoxitin	9	6
Erythromycin	5	3
Tetracycline	9	5
Imipenem	15	12
Ceftriaxone	10	4
Ciprofloxacin	16	10
Nalidixic Acid	3	3
Nitrofurantoin	9	2
Chloramphenicol	13	7

Table (5): Comparison of antibiotic significance among isolated intermediate hemolytic and non-hemolytic strains.

Antibiotics	No. of hemolytic intermediate strains	No. of non-hemolytic intermediate strains
Amoxicillin/Clavulanic acid	10	5
Trimethoprim/ Sulphamethoxazole	8	11
Cefoxitin	5	6
Erythromycin	13	7
Tetracycline	8	9
Imipenem	2	4
Ceftriaxone	6	10
Ciprofloxacin	3	5
Nalidixic Acid	6	11
Nitrofurantoin	10	7
Chloramphenicol	7	10

Many different antibiotic regimens have being subscribed at clinics making the correct choice difficult due to development of resistance and increase in opportunistic microorganisms. In addition, no justification of antibiotic prescription in the treatment of chronic, slowly-progressive forms of periodontal diseases are taken, which was also mentioned in previous studies [20, 21]. Recognizing specific types of periodontal infections can significantly influence the choice of antimicrobial treatment. Therapy should be tailored to differences in antibiotic susceptibility between various periodontal pathogens. The choice of antibiotic is empirical and based on the clinical signs.

Table (6): Comparison of antibiotic significance among isolated resistant hemolytic and non-hemolytic strains.

Antibiotics	No. of hemolytic resistant strains	No. of non-hemolytic resistant strains
Amoxicillin/Clavulanic acid	14	14
Trimethoprim/ Sulphamethoxazole	17	7
Cefoxitin	15	9
Erythromycin	11	11
Tetracycline	13	6
Imipenem	12	5
Ceftriaxone	13	7
Ciprofloxacin	11	5
Nalidixic Acid	20	7
Nitrofurantoin	10	12
Chloramphenicol	9	4

The most commonly prescribed antibiotic treatments for periodontitis are systemic antibiotic therapy for periodontal treatment which usually involves

monotherapy based on the β-lactams (amoxicillin with or without clavulanic acid), tetracyclines (tetracycline, doxycycline, and minocycline), clindamycin and ciprofloxacin. The obtained results from this study have concluded that the use of antibiotics in dental practice is characterized by prescription based on clinical and but not microbial susceptibility bacteriological diagnosis. In turn, an increased number of bacterial strains resistant to conventional antibiotics are found in the oral cavity in our study which relatively simulate a previous study by Roda *et al.* [22].

Microbial analysis should be used to determine the specific antimicrobial susceptibility pattern of the suspected pathogens, which can help in choosing the appropriate antibiotics, and may be followed-up with additional testing to verify the elimination or suppression of the putative pathogens. Unfortunately, for some clinicians, microbial analysis may be reserved for cases that are refractory to an initial course of antimicrobial therapy.

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Evaluation of the Effectiveness of Hepatitis B Vaccine among Vaccinated Adults in Misurata, Libya

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Abstract

Hepatitis B virus (HBV) is a blood-borne and sexually transmitted virus. Rates of new infection and acute disease are highest among adults, especially medical staff. Hepatitis B vaccination is the most effective measure to prevent HBV infection and its consequences particularly unvaccinated adults at increased risk for infection. This study was planned to evaluate the effectiveness of HBV vaccine among adults in Misurata city, Libya and to identify personal factors associated with serologic evidence of the immune response. This field trial study was conducted on 143 randomly selected adults attending Shohada Almakasba and Central Public Dental Clinic hospitals at Misurata city during the period from March 2013 to February 2014. They were classified into 3 catigories; 56 non health care worker adults (30 males and 26 females), 67 medical staff member (34 males and 33 females), in addition to twenty controls (10 males and 10 females) who did not receive any dose of hepatitis B vaccine). The hepatitis vaccine (Engerix ™ -B) used in Libyan vaccination centers, were chosen to vaccinate the participants. Serum samples for each participant were tested for the quantitative determination of anti-HBs antibody using Enzyme Linked Fluorescent Assay (Biomeureux). In our study, after vaccinating the studied group by three doses of HB vaccine, majority of them achieved a protective level of anti-HBs (84% and 86.3% for males and females respectively). While, only 16% of vaccinated males and 13.7% of vaccinated females showed immunoprophylaxis failure. The percentage of negative response to the first and second dose of hepatitis-B vaccine was 41% and 27% in males and 59.3% and 47.5% in females respectively. There is a statistical significant difference in the Mean (SD) of the expression of Hepatitis Bs antibodies titer after 1^{st} , 2^{nd} and 3^{rd} doses of vaccination from that of control group. There is a strong negative correlation between Hepatitis Bs antibodies titre after vaccination (1^{st} dose, 2^{nd} dose, 3^{rd} dose) and age. Also there is no statistical significant difference between health care workers and non health care groups as regard response to vaccination after1^{st}, 2^{nd} or 3^{rd} dose of vaccination. Finally, univariate analysis illustrated that there is a high statistical significant association between age and expression of Hepatitis Bs antibodies titre. While, other factors (gender, co-morbidity, parent vaccination, smoking habit); showed no significant association. These results suggested that Hepatitis B vaccination is the very effective measure to prevent HBV infection especially when given at younger age. All adults should be vaccinated by Hepatitis B vaccine with focusing on high risk groups.

Keywords: Hepatitis B virus; Vaccination; Antibodies, Univariate analysis.

1. Introduction

Hepatitis B is caused by the hepatitis B virus (HBV), an enveloped virus containing a partially double stranded circular DNA genome, and classified within the family hepadnaviridae [1]. Hepatitis-B virus infection is a global public health problem. It is estimated to have infected more than 2 billion people worldwide, of whom 400 million are chronically infected [2]. Every year there are over 4 million acute clinical cases of HBV, and about 25% of them remain carriers, 1 million people a year, die from chronic active hepatitis, cirrhosis or primary liver cancer [3]. Infection due to Hepatitis B virus results wide spectrum of liver diseases ranging from fulfillment hepatitis to cirrhosis and hepatocellular carcinoma. In addition, HBV carriers can transmit the disease for many years. Most infected people look perfectly healthy and have no symptoms of disease, yet they may be highly infectious [4].

Adult HBV infection is transmitted through contact with infected blood, semen or other body fluids primarily during sexual contact with an infected person or through other percutaneous or permucosal exposures. Approximately 5–10% of adults infected with HBV will develop chronic infection and 15% of those adults will develop chronic liver disease, including cirrhosis, liver failure, and liver cancer [5].

Hepatitis B vaccination is the most effective measure to prevent HBV infection and its consequences [6]. Safe and effective vaccines have been available since the early 1980 and in 1991 WHO recommended that all countries should introduce hepatitis B vaccination into their national immunization programs, by 1997 [7]. The Center for Disease Control and Prevention (CDC) and the Advisory Committee for Immunization Practices (ACIP) recommend universal vaccination for all children and adolescents, as well as adults who are at high risk for HBV infection [8]. Initial strategies for preventing HBV infection focused on 3-dose immunization of high-risk groups: health care personnel, men who have sex with men (MSM), injection drug users (IDU) and recipients of certain blood products [9].

Hepatitis B vaccination has resulted in a substantial decrease in the overall incidence of acute hepatitis B has declined 82%, from 8.5 per 100,000 in 1990 to 1.3 per 100,000 in 2008 [10]. In healthy population, 4–10% of vaccine recipients fail to produce protective levels of antibodies to the HBV vaccine after standard immunization [11]. Several non-genetic factors, including age, obesity, smoking, drug abuse, alcoholism, infections, immunesuppression and route of vaccination seem to be associated with

non-responsiveness [12]. Moreover, previous studies have demonstrated a possible genetic predisposition to hepatitis B vaccine non-responsiveness likely due to the presence of specific human leukocyte antigen (HLA) genotypes [13]. Specially HLA genotype DQ2 [14].

Due to little information about the effectiveness of hepatitis B vaccination in Misurata, therefore, this study was planned to throw light on the effectiveness of hepatitis B vaccine among vaccinated adults in Misurata.

2. Materials and Methods

2.1. Study design and setting

The study was conducted as a field trial on a group of adults in Shohadaa Almakhasba Clinic and Central Public Dental Clinic (CPDC) at Misurata city, Libya during the period from March 2013 to February 2014

2.2. Subjects

The study samples involved 143 randomly selected individuals. They were categorized as follow: Category 1: Composed of 56 non medical adults (30 males and 26 females). Category 2: Composed of 67 medical staff member (34 males and 33 females). All participants, within the first two categories, received three doses of recombinant hepatitis B vaccine. Serum samples were collected at different post vaccination intervals following the completion of first, second and third dose of hepatitis B vaccine. Category 3: Twenty controls (10 males and 10 females), who did not receive any dose of hepatitis B vaccine. An informed consent was obtained from all participants in this study.

2.3. Methods

2.3.1. Questionnaire

The studied group was interviewed by a pre-constructed questionnaire containing data about age, sex, occupation, smoking habit, co-morbidity and parent's vaccination.

2.3.2. Hepatitis - B vaccine

The hepatitis vaccine (Engerix ™ -B) used in Libyan vaccination centers. It is a rDNA hepatitis-B vaccine; manufactured by GSK Biologicals s. a. Rixensart, Belgium, and is approved by WHO. The vaccine was stored at + 2 to + 8°C and injected intramuscularly at 0, 1, 2 months duration.

2.3.3. Blood samples for serological analysis

About 3 ml of venous blood were collected from each participant. The sera were removed from the tubes after clotting and centrifugation. Serum samples were divided into three labeled sterile tubes to avoid repeated freezing and thawing. Serum samples were stored frozen at -20°C in the Ibn senaa laboratory before being analyzed.

2.3.4. Kit for detection of Hepatitis B surface antibody (HBsAb) level

Vidas Anti-HBs Total Quick is an automated quantitative test for use on the mini VIDAS instrument, for the immunoenzymatic detection of antibodies to hepatitis B surface antigen (anti-HBs) in human serum or plasma using ELFA technique (Enzyme Linked Fluorescent Assay "Biomeureux").

2.3.5. Detection of Hepatitis B surface antibody (HBsAb) level

The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The solid phase receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready to use and are pre-dispensed in the sealed reagent strips. (According to instruction manual Manuel of the provided company). The five reaction steps are performed automatically by the instrument. The intensity of the fluorescence is proportional to the quantity to anti-HBs in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

Figure (1): MiniVIDAS, Biomeruex France.

Figure (2): Mini VIDAS, ELFA Strips

Figure (3): Centrifuge, Hettich, D- 78532 Tuttlingen, Germany

2.3.6. Quality control

One positive control and negative control are included in each VIDAS Anti-HBs Total Quick kit.

2.3.7. Result and interpretation

The final result appears on the result sheet expressed in mlU/ml. the measuring range is 5-500 mlU/ml. VIDAS Anti-HBs Total Quick is calibrated against the international standard (WHO 1st reference preparation 1977) [15].

Interpretation of results was as follows: Titer <8 mlU/ml was considered negative result; Titer 8-12 mlU/ml was considered intermediate result; and Titer >12 mlU/ml was considered positive result. If an indeterminate result is obtained, a second specimen collected at a later date.

2.3.8. Statistical analysis of the results

The collected date was analyzed by SPSS software version 18 and the statistical analysis was performed using Chi-square and student's test. Logistic regression models were used to assess the relationship between vaccine response and variables. Results were considered significant when $P< 0.05$.

3. Results and Discussion

In the present study, a total of 143 cases were randomly selected from Shohadaa Almakhasba Clinic and Central Puplic Dental Clinic (CPDC) at Misurata city during the period from March 2013 to February 2014.

Patients included in this study were distributed according to the selected groups, age and sex were presented in Table (1), which showed that group-one included 56 adult non medical individuals (30 males and 26 females), group-two included 67 adult medical staff individuals (34 males and 33 females) and group-three included 20 adult control individuals (10 males and 10 females).

Table (1): Distribution of individuals among the studied groups according to age and gender.

Group distributions	Age group								Total
	21 – 30 years				> 30 years				
	Male		Female		Male		Female		
	No.	%	No.	%	No.	%	No.	%	
Non-medical staff	23	41.1	11	19.6	7	12.5	15	26.8	56
Medical staff	25	37.3	22	32.8	9	13.4	11	16.5	67
Control	6	30.0	4	20.0	4	20.0	6	30.0	20
Total	54	37.7	37	25.9	20	14.0	32	22.4	143

N.B. Groups were comparable as regard age and gender distribution. There was no statistical significant difference (P > 0.05).

Distribution of some adult cases included in the study according to disease status was presented in Table (2), which showed a total 37 cases (30.1 %), eight cases suffering from chest infection (4males and 4 females). Other nine cases with diabetes mellitus (2 males and 7 females). Finally 20 cases suffering from overweight (13 males and 7 females).

Table (2): Distribution of some adult cases according to disease state.

Disease state	Sex				Total
	Male		Female		
	No	%	No	%	
Chest infection	4	3.2	4	3.2	8
Diabetes mellitus	2	1.6	7	5.7	9
Over weight	13	10.6	7	5.7	20
Total	19	15.4	18	14.6	37

The most prevalent health problems among the studied cases were over weight among males (10.6 %) and diabetes mellitus among females (5.7 %).

The effects of number of administered vaccine doses on seropositivity to HBs antibodies in adult male and female groups are illustrated in Tables (3) and (4) and Figures (1) and (2). The results showed that through vaccination by three doses of HB vaccine, majority of the adult male group (84.0%) achieved a protective level of anti-HBs and only 16% of the vaccinated adult males showed immunoprophylaxis failure. The percentage of negative response to the first and second dose of hepatitis-B vaccine was 41% and 27% respectively. However in case of adult female group, the results indicated that vaccination by three doses of HB vaccine, (86.3%) achieved a protective level of anti-HBs and only 23.7% of the vaccinated adult females showed immunoprophylaxis failure. The percentage of negative response to the first and second dose of hepatitis-B vaccine was 59.3% and 47.5% respectively.

Table (3): Effect of number of administered vaccine doses on seropositivity to HBs antibodies in adult male group.

Seropositivity to antiHBs	1st dose		2nd dose		3rd dose	
	No.	%	No.	%	No.	%
Negative	35	59.3	28	47.5	14	23.7
Weakly positive	5	8.5	5	8.5	8	13.6
Positive	19	32.2	26	44.0	37	62.7
Total	59	100	59	100	59	100

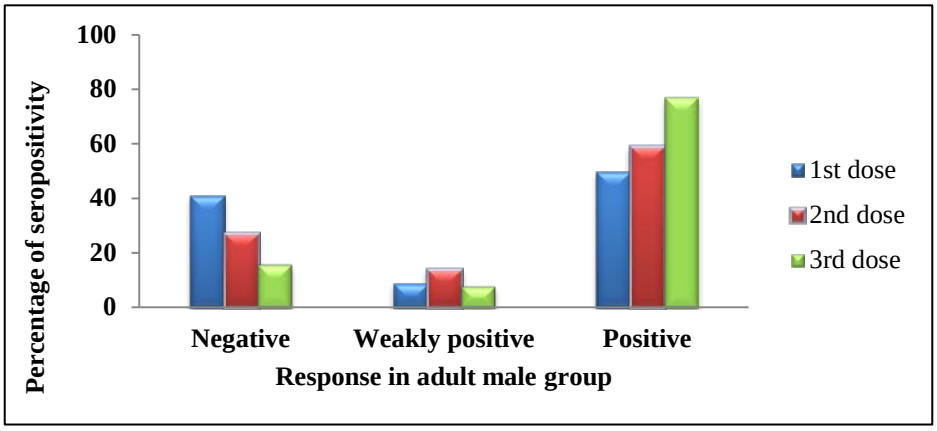

Figure (1): Effect of number of administered vaccine doses on seropositivity to HBs antibodies in adult male group.

Statistical analysis of the difference in the Mean (SD) of the expression of Hepatitis Bs antibodies titre among adult vaccinated groups after 1st and 2nd vaccination doses from that in control group**,** proved that there is high statistical significant difference in the expression of Hepatitis Bs antibodies

titre between vaccinated and control group after 1st and 2nd doses of vaccination. Meanwhile, there is no statistical significant difference between medical and non-medical group Table (5).

Table (4): Effect of number of administered vaccine doses on seropositivity to HBs antibodies in adult female group.

Seropositivity to antiHBs	1st dose		2nd dose		3rd dose	
	No.	%	No.	%	No.	%
Negative	26	41	17	27	10	16
Weakly positive	6	9	9	14	5	8
Positive	32	50	38	59	49	76
Total	64	100	64	100	64	100

Table (5): Difference in the Mean (SD) of the expression of Hepatitis Bs antibodies titer among adult vaccinated groups after 1st and 2nd vaccination doses from that of control group.

Hepatitis Bs Ab titre	Vaccinated groups		Control Group	Kruskal Wallis test	*P* value
	Adult Non-Medical Group	Adult Medical Staff Group			
	N (56)	N (67)	N (20)		
After 1st dose					
Mean ± (SD)	247.7 (240)	161.7 (216.7)	4.0 (0.1)	χ^2 = 20.06	< 0.001
Median	232	4	4		
After 2nd dose					
Mean ± (SD)	277.9 (238.3)	239.1 (235.6)	4.0 (0.1)	χ^2 = 31.03	< 0.001
Median	500	136	4		

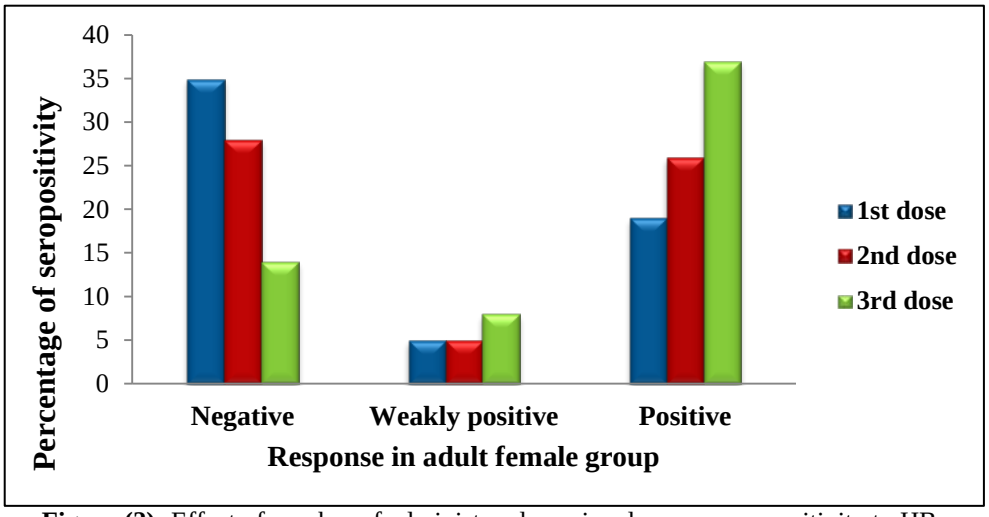

Figure (2): Effect of number of administered vaccine doses on seropositivity to HBs antibodies in adult female group.

Table (6) showed that statistical analysis of the difference in the Mean (SD) of the expression of Hepatitis Bs antibodies titre after 1st, 2nd and 3rd doses of vaccination from that of control group**,** and the results indicated that there is high statistical significant difference in the expression of Hepatitis Bs antibodies titre between vaccinated and control group after 1st dose, 2nd dose and 3rd doses of vaccination.

Table (6): Difference in the Mean (SD) of the expression of Hepatitis Bs antibodies titre after 1st, 2nd and 3rd dose of vaccination from that of control group.

Hepatitis Bs Ab titer	**Vaccinated Group**	**Control Group**	**Mann Whitney test**	***P*-value**
After 1st dose				
Number (143)	123	20		
Mean ± (SD)	200.9 (230.7)	4.0 (0.1)	Z = - 4.19	< 0.001
Median	21	4		
After 2nd dose				
Number (143)	123	20		
Mean ± (SD)	256.8 (236.6)	4.0 (0.1)	Z = - 5.39	< 0.001
Median	250	4		
After 3rd dose				
Number (300)	280	20		
Mean ± (SD)	189.0 (216.4)	4.0 (0.1)	Z = - 6.52	< 0.001
Median	49	4		

Statistical analysis of the results showed that there was no statistical significant difference between both adult male group and female group as regard response to vaccination after1**st**, 2**nd** or 3**rd** dose of vaccination; so there is no clear effect of the gender on the response Table (7).

Table (7): Difference in the Mean (SD) of the expression of Hepatitis Bs antibodies titer by gender

Hepatitis Bs Ab titer	**Male**	**Female**	**Mann Whitney test**	***P*-value**
After 1st dose				
Number (123)	64	59	Z = - 1.94	0.052
Mean ± (SD)	240.8(235.7)	15 7.6 (219.1)		
Median	157	400		
After 2nd dose				
Number (123)	64	59	Z = - 1.516	
Mean ± (SD)	284.9 (232.2)	22 6.3 (239.6)		0.13
Median	454.5	77		
After 3rd dose				
Number (280)	146	134	Z = - 0.731	
Mean ± (SD)	20 2.3 (220.9)	17 4.5 (211.2)		0.465
Median	55	41.5		

The results of studying the effect of age on immune response to each hepatitis-B vaccine in adult male and female groups, were illustrated in Tables (8) and (9) and in Figures (3) and (4) and the results proved that there is a higher expression of Hepatitis Bs antibodies titer after (full vaccination) among those (21- 30) years than that in other age groups.

Table (8): Effect of age on immune response of adult males to each vaccine dose.

Immune response	1st dose				2nd dose				3rd dose			
	21-30		>30		21-30		>30		21-30		>30	
	No	%	No	%	No	%	No	%	No	%	No	%
Negative	16	33.3	10	62.5	16	20.8	7	43.7	6	12.5	4	25.0
Weakly positive	4	8.3	2	12.5	4	14.6	2	12.5	2	4.2	3	18.7
Positive	28	58.4	4	25.0	28	64.6	7	43.7	40	83.3	9	56.3
total	48	100	16	100	48	100	16	100	48	100	16	100

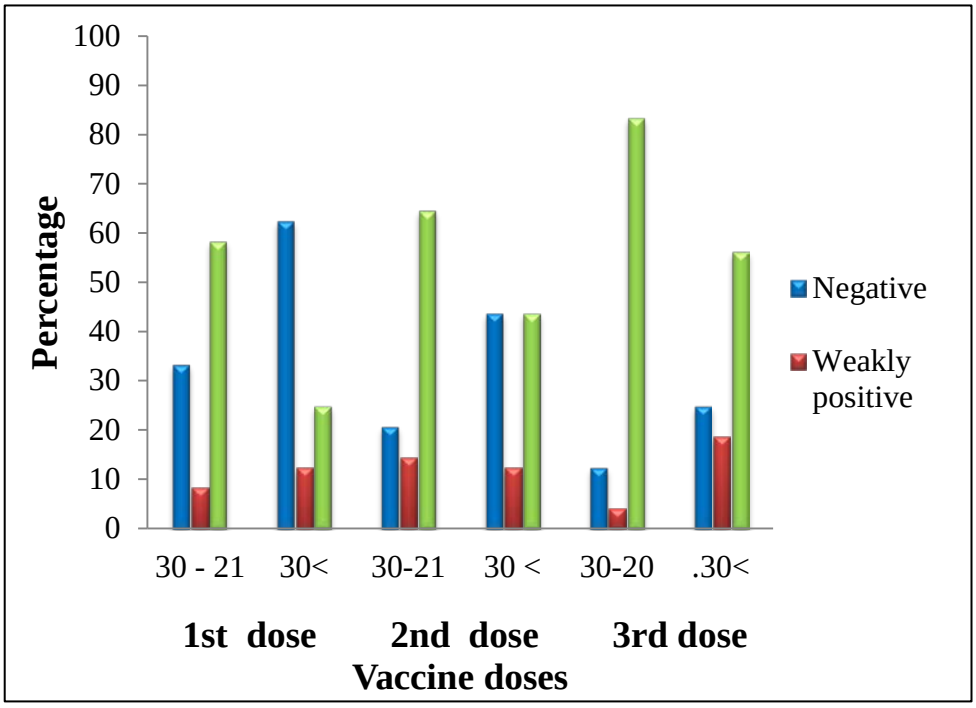

Figure (3): Effect of age on immune response of adult male to each vaccine dose.

Table (9): Effect of age on immune response of adult females to each vaccine dose.

Immune response	1st dose				2nd dose				3rd dose			
	21-30		>30		21-30		>30		21-30		>30	
	No	%	No	%	No	%	No	%	No	%	No	%
Negative	11	33.3	24	92.2	9	27.3	19	73.1	3	9.1	11	42.3
Weakly positive	4	12.1	1	3.8	1	3.1	4	15.4	3	9.1	5	19.2
Positive	18	54.6	1	3.8	23	69.6	3	11.5	27	81.8	10	38.5
Total	33	100	26	100	33	100.	26	100	33	100	26	100

There is statistical significant negative correlation between Hepatitis Bs antibodies titre after vaccination (1st dose, 2nd dose & 3rd dose) and age. While, there is statistical significant positive correlation between Hepatitis Bs antibodies titre after vaccination 1st dose with those of 2nd dose and 3rd dose Table (10).

Table (10): Spearman's rho Correlation between the Hepatitis Bs Ab titre after vaccination (1st dose, 2nd dose, 3rd dose) and Age

Characteristics	Correlation coefficient (r)	*P*-value
Hepatitis Bs Ab titer after 1st dose Vs:Age	- 0.573	< 0.001
Hepatitis Bs Ab titer after 2nd dose	0.911	< 0.001
Hepatitis Bs Ab titer after 3rd dose	0.735	< 0.001
Hepatitis Bs Ab titer after 2nd dose Vs:Age	- 0.534	< 0.001
Hepatitis Bs Ab titer after 3rd dose	0.861	< 0.001
Hepatitis Bs Ab titer after 3rd dose Vs:Age	- 0.31	< 0.001

There is high statistical significant difference in the expression of Hepatitis Bs antibodies titre after 1st, 2nd and 3rd vaccination doses among adult vaccinated group. Table (11).

Table (11): Difference in the Mean (SD) of the expression of Hepatitis Bs Ab titer among adult vaccinated group after 1st, 2nd and 3rd vaccination dose.

Hepatitis Bs Antibodies titre	Adult vaccinated group (123)			Friedman Test	P-value
	after 1st vaccination dose	after 2nd vaccination dose	after 3rd vaccination dose		
Mean ±(SD)	200.9(2307)	256.8(236.6)	333.8(217)	χ^2 = 107.03	<.0001
Median	21	250	500	3	

Finally**,** Univariate analysis for association between the expression of Hepatitis Bs antibodies titre and (Age groups, gender, co-morbidity, parent

vaccination, smoking habit); illustrated that there is a high statistical significant association between age group and expression of Hepatitis Bs antibodies titre. While, other factors showed no significant association Table (12).

Table (12): Univariate analysis for association between the expression of Hepatitis Bs Ab titre and factors (Age groups, gender, co-morbidity, parent vaccination, smoking habit).

Source	Type III Sum of Squares	Df	Mean Square	F	*P*
Corrected Model	555566.489[a]	3	185188.830	4.169	0.007
Intercept	405566.049	1	405566.049	9.129	0.003
Age groups	4665271.908	3	1555090.636	50.918	0.000 *
Co-morbidity	20720.582	3	6906.861	0.149	0.9300
Gender	54197.260	1	54197.260	1.183	0.2780
Father Vaccination	23656.033	1	23656.033	0.532	0.4660
Mother Vaccination	41111.296	1	41111.296	0.925	0.337
Surgery	10960.458	1	10960.458	0.634	0.427
Dentist	2051.983	1	2051.983	0.119	0.7310
Corrected Total	1.371E7	299			

[a] R Squared = .041 (Adjusted R Squared = .031) General linear model was used

* There is a high statistical significant association between age group and expression of Hepatitis Bs Ab titre. While, other factors showed no significant association.

HBV infection is preventable with safe and effective vaccines that have been available since 1982. The vaccine is 95% effective in preventing chronic infections from developing countries, and is the first vaccine against a major human cancer. More than 160 countries have already added this vaccine to their routine immunization programs [16]. The schedule adopted by Ministry of Health was three doses of yeast-recombinant hepatitis B vaccine administered to all infants at 2, 4, 6 to coincide with other compulsory vaccines (Diphtheria, Tetanus, Pertussis and oral polio (DPT-OPV) [17].

This study included 143 healthy adult individuals, who were divided into three groups. Group (A) included 56 non medical adult, while group (B) included 67 medical staff member, and group (C) included 20 controls (they did not receive any dose of hepatitis B vaccine). To the best of our knowledge, this is the first study in Libya and provides the local epidemiological data assessing the immune response to HBV vaccine among adults.

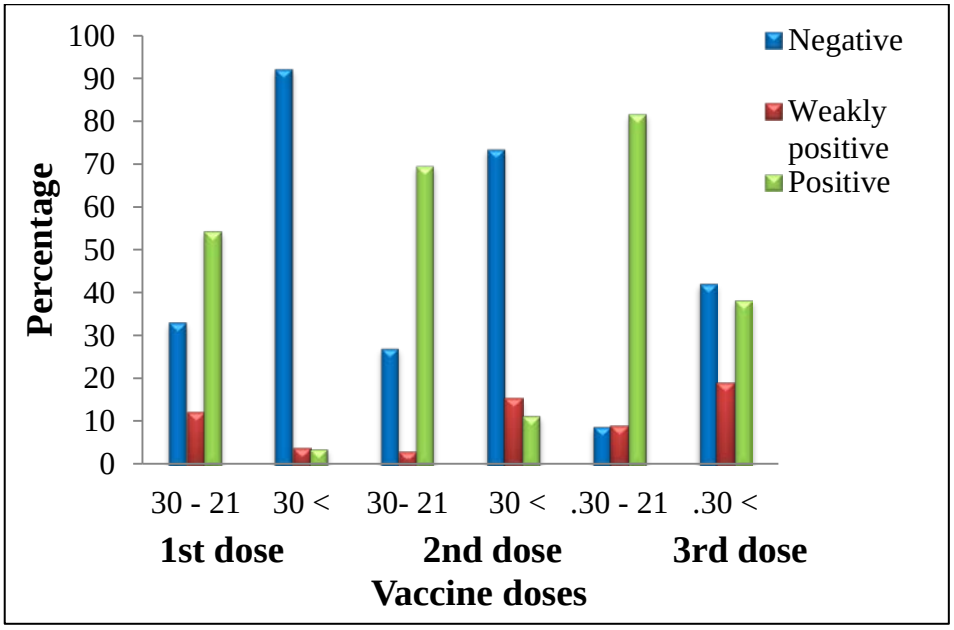

Figure (4): Effect of age on immune response of adult female group to each vaccine dose.

The effects of number of administered vaccine doses on seropositivity to HBs antibodies in adult male and female groups in the current study showed that through vaccination by three doses of HB vaccine, majority of the adult male group (84.0%) achieved a protective level of anti-HBs and only 16% of the vaccinated adult males showed immunoprophylaxis failure. The percentage of negative response to the first and second dose of hepatitis-B vaccine was 41% and 27% respectively. However in case of adult female group, the results indicated that vaccination by three doses of HB vaccine, (86.3%) achieved a protective level of anti-HBs and only 23.7% of the vaccinated adult females showed immunoprophylaxis failure. The percentage of negative response to the first and second dose of hepatitis-B vaccine was 59.3% and 47.5% respectively. Statistical analysis of the results showed that there was no statistical significant difference between adult males and adult females as regard response to vaccination after 1st, 2nd or 3rd doses of vaccination; so there is no clear effect of the gender on the response. However, statistical analysis of the results indicated that there is statistical significant positive correlation between Hepatitis Bs antibodies titre after vaccination 1st dose with those of 2nd dose and 3rd dose.

The current result is consistent with the findings obtained from Kuhil *et al.* [18], study which noted that there is no significant difference of results

regarding sex. Also the previous studies conducted by Karaglu *et al.,* and Sallam *et al.,* which revealed that there was no difference in results according to the sex [19, 20]. On the contrary to our findings, the study conducted by Wildgrub *et al.,* found that there was a high anti-HBs antibody concentrations occurred significantly more frequently in females than in males [21]. While, McMahon *et al.,* stated that initial anti-HBs level, older age at vaccination and male sex were associated with persistence of higher anti-HBs levels at 15 years [22].

In this study, the results of studying the effect of age on immune response to each hepatitis-B vaccine in both adult males and females, proved that there is a higher expression of Hepatitis Bs antibodies titre after (full vaccination) among those (21- 30) years than that in other age groups. Also, there is a statistical significant negative correlation between Hepatitis Bs antibodies titer after vaccination (1^{st} dose, 2^{nd} dose, 3^{rd} dose) and age.

The findings are more or less in the agreement with earlier reports. Roome *et al.,* also observed the inadequate levels of antibodies in relation to increasing age, from 2.8% among those younger than 30 years to 42.1% among those older than 60 years of age [23].

The observation favours the hypothesis that with increasing age seroprotective antibody formation after vaccination is decreased. This finding is of great clinical significance as non-responders remain susceptible to HBV infections. Therefore, from infection control perspective, the post vaccination HBsAb levels should be determined for all persons susceptible to HBV infections.

Medical staff group (health care workers) are at high risk of acquiring HBV and non responder's rates after HBV vaccination were not reported previously in Libyan health care workers. Therefore we evaluated immune response to HBV vaccine in health care workers at a Shohada Almakasba Clinic and Central Public Dental Clinic in Misurata, Libya. The results of the present study indicated that there was no statistical significant difference between health care workers and non health care workers group as regard response to vaccination after 1^{st}, 2^{nd} or 3^{rd} dose of vaccination.

Statistical analysis of the difference in the Mean (SD) of the expression of Hepatitis Bs antibodies titer among adult vaccinated groups after 1^{st} and 2^{nd} vaccination doses from that in control group**,** proved that there is high statistical significant difference in the expression of Hepatitis Bs antibodies

titer between vaccinated and control group after 1st and 2nd doses of vaccination. Univariate analysis for association illustrated that there is a high statistical significant association between age and expression of Hepatitis Bs antibodies titre. While, other factors (gender, co-morbidity, parent vaccination, smoking habit) showed no significant association.

Host factors that may affect hepatitis B vaccine response in the current study included age of 30 years or greater. Immunological response to Hepatitis B is dictated by several host characteristics that influence the body's ability to mount an effective defense. In particular, immune response to the vaccine is dependent on the activity of T-cells and may be inhibited by conditions associated with impaired T-cell function [24]. Factors associated with failure to develop hepatitis B seroprotection include older age, lack of immunocompetence, smoking habits, and genetics [25].

The results obtained from previous studies support the notion that the efficacy of hepatitis B vaccine is variable from one study to another, this could be attributed to many reasons including: prevalence of hepatitis B disease "endemicity", vaccination coverage, vaccination schedule, variability in vaccine synthesis or preparation, defect in vaccine cold chain and differences in methods used to evaluate antibody titer [26].

Sjogren stated that the distinction between true nonresponse (after adequate immunization) and waning anti-HBs levels is important. Data from subjects with waning anti-HBs levels show that immunologic memory may still protect these individuals against acute HBV infection or may prevent chronic infection with HBV for $\leq$ 10 years after immunization [27]. Other investigators still raise the possibility for a booster dose. while, Wang *et al.*, cleared that their previous study suggests that routine booster vaccination may not be necessary to provide protection against chronic HBV infection [28].

4. Conclusions

The effectiveness of Hepatitis B vaccine in adults is very high (84.0%) and (86.3%) for adult males and females respectively, with no statistical significant difference in antibody level among both genders either in medical and non-medical individuals. A high seroprotection rate was elicited by hepatitis B vaccination especially in younger age (21- 30 years) with a trend of decreasing antibody level by increasing age.

Recommendations

The recommendations which have emerged from this study: 1) continue the hepatitis B universal immunization and increase the vaccine coverage to 100%; 2) implement the immunization policy that recommends hepatitis B immunization for adults who are not previously vaccinated in early childhood; 3) continuously monitor the program and ensure cold chain preservation; 4) future studies should be conducted to cover vaccination of other groups and in particular who are exposed to occupational hazards.

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Histopathological Findings in Libyan Women with Postmenopausal Bleeding

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Abstract

postmenopausal bleeding is likely due to a benign cause in woman of postmenopausal age, but this bleeding is the most common presenting symptom of endometrial cancer and should always be regarded with suspicion and must be evaluated at the appointed time. to investigate the histopathological causes of postmenopausal bleeding, and estimate the prevalence of malignancy in patients have presented with postmenopausal bleeding. we investigated the histopathological findings in women presented with postmenopausal bleeding by conducting a three-year retrospective study of gynecological surgical specimens from 110 women accessed in the Department of pathology at Misurata Teaching Hospital. the mean age for women with postmenopausal bleeding was 56.7 ± 9.8 years old and 80.9% of them had benign pathology. Endometrial polyp was the commonest pathology representing 46.4% of the total specimens while endometrial atrophy representing 10.9%. Of the total cases; 5.5% had simple cystic endometrial hyperplasia. Leiomyoma was reported in 10% of the cases associated with other pathology including chronic nonspecific cervicitis and adenomyosis. Three patients (2.7%) were diagnosed to have premalignant changes. The frequency of malignancy among these women was 11.8 %, most of them reported histopathologically to have endometrial carcinoma. benign pathology is more frequent cause of postmenopausal bleeding, but we must always evaluate and rule out malignancy by oriented biopsy.

Keywords: Postmenopausal bleeding, Histopathological studies**,** endometrial carcinoma

1. Introduction

Postmenopausal bleeding (PMB) is essentially the bleeding of most importance at the postmenopausal age as an alarming sign that may be associated with uterine malignancy. So; all postmenopausal women with uterine bleeding patients should be evaluated for endometrial carcinoma. Vaginal bleeding in the postmenopausal years is usually attributed to an intrauterine source, it occurs in 4- 11% of postmenopausal women [1-4] and the incidence appears to be correlated with time since menopause. The differential diagnosis of bleeding in postmenopausal women is narrower than that of abnormal bleeding in premenopausal

women due to the lack of the variable influence of ovarian hormones. PMB is often caused by abnormalities of the endometrium, whether they are benign or malignant. Endometrial atrophy is the most common endometrial finding accounting for 60–80% in women with PMB [5]. PMB is the primary symptom of endometrial cancer and 10%–15% of such bleeding has endometrial carcinoma [6-10]. In contrast, the prevalence of endometrial polyps in patients with PMB and an increased endometrial thickness measured with transvaginal sonography is estimated to be around 40% [11, 12]. There is little data concerning about this issue in our locality. So, a thorough work-up is needed for the women presenting with PMB especially to rule out malignancies in a defined geographical area. Our objective was to investigate the histopathological causes of postmenopausal bleeding, and to estimate the prevalence of malignancy in patients presenting with PMB.

2. Methodology

We investigated the histopathological findings in all consecutive patients presented with PMB and managed in the of Department of gynecology at Misurata Teaching Hospital; by conducting a three-year retrospective study gynecological surgical specimens which belonged to 110 women. These 110 women had assessed in the Department of Histopathology during the period between January 2003 and December 2005. The records of 110 patients with PMB including 2 patients have had subtotal abdominal hysterectomy, were reviewed and assessed. Out of them, 73 (66.4%) were from biopsy specimens including one vulval biopsy and 37 (33.6%) from hysterectomy specimens with or without salpingo-oophorectomy. Demographic, medical and gynecological data of these patients were assessed including patient's age, parity, the interval between menopause, history of hormonal therapy, onset of PMB, and biopsy site. All women presenting with vaginal bleeding underwent transvaginal ultrasound scanning to evaluate the endometrium as part of their routine assessment and the double wall endometrial thickness was measured. The histopathologic reports were reviewed, and identifying the causes of PMB which were diagnosed by histopathological examination of gynecological specimens submitted to the department of pathology. The available data were investigated and statistical comparison has made between the groups where appropriate, test of difference of proportions using Z-score statistics at 5% of significance was used.

3. Results and Discussion

The youngest patient was 45 years old while the oldest one was 85 years old, with a mean age of 56.7 ± 9.8 years old. Majority of the patients (84.5%) were aged 50 years old or more, patient's age shown in Table (1), this also shows patient's distribution in relation to their parity revealing that more than three-fourths of the patients were highly praous women (para ≥ 6). The interval between menopause and onset of PMB ranged between 1 and 21 years with a mean of 4.9 ± 3.8 year. The onset of PMB was seen more often in the earlier postmenopausal years i.e. < 6 year (68%). Its frequency decreased as time goes by.

Table (1): Distribution of PMB according to age and parity

Parameter	No. of patients	Frequency
Age group		
40-49	17	15.5%
50-59	56	50.9%
60-69	24	21.8%
≥ 70	13	11.8%
Total	110	100%
Parity		
P0	1	0.9%
P1-5	24	21.8%
P6-10	60	54.4%
P≥ 11	25	22.7%
Total	110	100%

Nearly third of the postmenopausal women were diabetic (34.5%) and hypertensive (28.2%) as shown in Table (2); while Table (3) shows the gynecological diseases associated with the PMB. Of the 110 postmenopausal patients; 89 (80.9%) had benign pathology and few patients had no evidence of postmenopausal uterine pathology (4.5%) as seen in Figure (1). There were 3 (2.7%) patients with premalignant changes. Approximately, in 50% of the specimens there was more than one pathological finding.

The prevalence of malignancy among women with postmenopausal bleeding was found to be 11.8 %; and most of them reported histopathologically to have endometrial cancer (84.6%) included one post subtotal hysterectomy. Table (4) shows the pathological causes of PMB. Postmenopausal endometrial polyp was the most common benign entity representing 41% of the cases.

Further investigations of postmenopausal women with endometrial polyps revealed association of chronic nonspecific cervicitis in 22.2% of these patients and 8.9% associated with adenomatous cervical polyp.

Table (2): Medical disorders among patients with PMB

Medical disorder	No. of cases	Frequency
Diabetes Mellitus	38	34.5%
Hypertension	31	28.2%
Anemia	12	10.9%
Urinary tract infection	9	8.2%
Bronchial Asthma	3	2.7%
Cardiac diseases	2	1.8%
Hypothyroidism	1	0.9%

Table (3): Gynecological diseases in patients with PMB

Gynecological diseases	No. of cases	Frequency
Pelvic inflammatory diseases	16	14.5%
Vagina wall Prolapse	10	9.1%
Uterine Fibroid	7	6.4%
Cervical Polypus	6	5.5%
Ovarian tumor	4	3.6%
Urine incontinence	8	7.3%

Regarding endometrial cancer; the evaluation of these patients revealed that the age of them was ranged between 50 & 85 years old with a mean age of 62.5 ± 10.4 years. The prevalence of endometrial cancer was increased with older age as seen in Table (5); while reported 7.1% in those aged 50 to 59 years it reached 16.7% in those aged 60 to 69 years and 23.1% in patients aged 70 years or more, but the differences between these 3 age groups were not significant (P > 0.05).

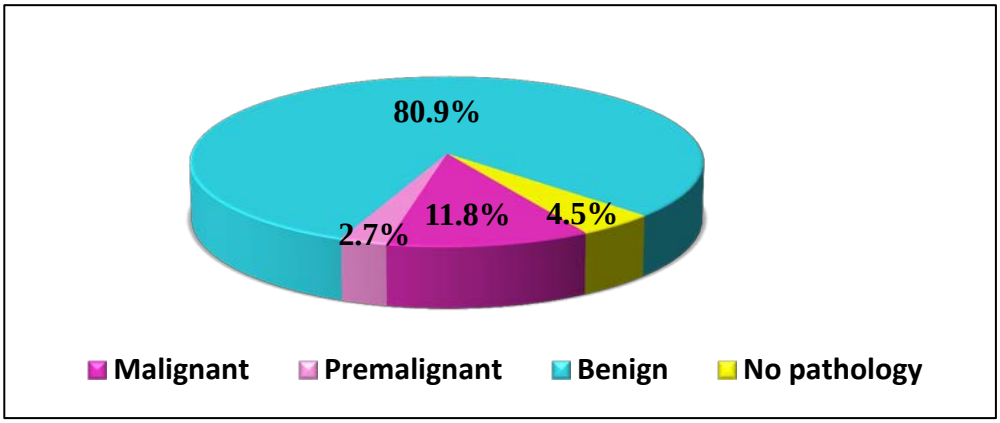

Figure (1): Types of lesions causing PMB

Postmenopausal bleeding (PMB) is a common clinical problem and approximately 10% of women experience this problem [13]. Moreover, 25% of gynecologic surgeries reported to be indicated for abnormal uterine bleeding [14]. It should always be taken seriously and investigated, as it could be a sign of endometrial carcinoma, which has a much higher cure rate if diagnosed early. So; the awareness of the serious implications of PMB among patients and physicians resulted in an earlier detection of malignancy. In our study, only 15.5% of the postmenopausal women were under the age of 50 years included from the age of 45–49 years. We did not have reports regarding average age of menopause among our population. The mean age at presentation with vaginal bleeding was 56.7 ± 9.8 years which is younger compared with other reports. [15-17].

Table (4): Histopathological findings of Postmenopausal bleeding

Type of pathology	No. of cases	Frequency
Endometrial cancer	11	10.0%
Cervical carcinoma	1	0.9%
Adenomatous endometrial polyp	45	40.9%
Endometrial atrophy	12	10.9%
Cervical dysplasia	2	1.8%
Cervical polyp	6	5.5%
Ovarian cancer	1	0.9%
Leiomyoma	10	9.1%
Endometrial hyperplasia	7	6.4%
Chronic nonspecific ulcerous cervicitis	4	3.6%
Tuberclous endmetritis	1	0.9%
Adenomyosis	3	2.7%
No organic causes	5	4.5%
Others	2	1.8%
Total	110	100.0%

Table (5): Endometrial cancer and age

Age group	Number of patients	Frequency of endometrial cancer
40-49	17	0
50-59	56	4 (7.1%)
60-69	24	4 (16.7%)
≥ 70	13	3 (23.1%)
Total	110	11(10%)

Usually, PMB occurs in early years of menopause and is less frequent after 3 or more years of menopause [13]. We found that 68% of the patients had the bleeding in the earlier postmenopausal years less than 6 years. Increasing time interval between menopause and onset of postmenopausal bleeding is highly indicative of malignancy.

Benign lesions as a cause of PMB were found in approximately 81% of the cases, which comparable with the incidence (84%) reported by Dawood N.S. *et al.* (15); but inconsistent with Kauser *et al.* [18] who reported only 48%. Amongst benign causes; endometrial polyp was the most common in our study accounting for 41% of patients with PMB and this was in agreement with previous studies [10, 12], while Kauser *et al* [18], reported a much lower prevalence than the current series (12%). Atrophic endometritis was responsible for 10.9% of the cases of PMB, while it is reported to be two times more (21.2%) by Dawood *et al* [15]. The present study showed that leiomyoma was reported to be four times (9.1%) more prevalent as compared with the previously mentioned study (1.9%). This could be explained by older mean age of their patients (63.6±9.3 years) as leiomyomas usually shrink after menopause.

The reported incidence of endometrial carcinoma in women presenting with PMB varies widely between different studies, from 1% to 24%. [7, 19–24]. The prevalence found in our study (11.8% inclusive of carcinoma endometrium, cervix, and ovary) occupies an average position when compared with the previously mentioned studies and much lower than reported by Wonderossen Ergette [25] from Ethiopia 60.8%, Liaquat *et al* [26], 53.7%, Asif *et al* [27], 44%, Kauser *et al* [18], 30%, and Ghazi *et al* [28], 20%. It is difficult to know the true prevalence of malignancy in PMB in our society because many patients may not to seek for medical advice at this age. Also, the lack of education and awareness of the patients, and absence of screening programmes. It is acceptable for the incidence of endometrial cancer to vary between different populations, depending on the presence of risk factors for endometrial malignancy. Our result regarding incidence of endometrial carcinoma (10%) is comparable with Valerie [13], Youssef *et al* [29], and Escoffery *et al* [6]; while it is inconsistent with other previous reports [15, 18]. The wide range in the observed incidence of endometrial carcinoma may have contributed to the variation in the selection criteria used and also the prevalence of risk factors for endometrial carcinoma. The risk of developing endometrial cancer increases with age [30] and the same result was observed in our study. Although endometrial carcinoma increased in older age groups and showed peak prevalence (23.1%) in patients aged 70 years or more, but these increases not reached the level of significancy. This finding is also consistent with Gredmark *et al* [7]. Postmenopausal bleeding is not an uncommon event. While it is not always a symptom of cancer, it should be evaluated whatever the amount of bleeding. The exclusion of endometrial hyperplasia and carcinoma is the key issue in the evaluation of patients with such abnormal uterine bleeding.

4. Conclusions

Postmenopausal endometrial polyp was the most common benign entity. Despite the fact that benign pathology is more frequent than malignancy as a cause postmenopausal uterine bleeding; but it must always rule out a cancer by oriented biopsy.

Recommendations

The reported prevalence of malignancy in women with postmenopausal bleeding is considerably high suggesting a screening program going hand to hand with public education for postmenopausal women in an attempt to prevent, detect and manage health problems in our mothers and improve their quality of life.

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Synthesis and Antifungal Study of Some Azo Cresols

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Abstract

Three azo cresol derivatives consisting of anthracene moiety were synthesized by coupling 9-anthryldiazonium slat with *o*-, *m*- and *p*-cresols. The synthesized compounds were screened for their antifungal activities against *Aspergillus niger* (*A. niger*). The results were compared with two commercially available antibiotics and showed moderate to high levels in inhibitory.

Keywords: Azo cresols, synthesized, screened, antifungal activities, *Aspergillus niger*.

1. Introduction

Phenolic derivatives have been reported to have the capability of inhibiting the growth of some types of bacteria and fungi. The antifungal properties of the phenolic derivatives that were extracted from olive pomace, a by-product of olive oil production, against *Alternaria solani, Botrytis cinerea* and *Fusarium culmorum* was studied. Such naturally occurring phenolic compounds showed good antifungal properties against the growth of the mentioned above three fungi [1]. Large production of azo derivatives is produced globally due to the high demand for these derivatives by various industries [2]. The use of azo dyes has increased in foods, pharmaceutical and in textile industries due to their biological activity which gives azo dyes the potential to inhibit the growth of microorganisms. The microorganisms cause a degradation of azo dyes and leading to the formation of harmful aromatic amines [3-5]. The antimicrobial properties of the azo dyes have been intensively investigated by a number of research groups. These studies involved the possibility of such dyes to inhibit the growth of bacteria and fungi [6,7]. Some phenolic azo compounds derived from the salicylic acid exhibited levels of resistivity against the fungi growth on jowar seeds [8]. Other azo dyes have been synthesized and tested against the fungi that harms some types of seeds. It has been reported that the synthesized phenolic azo derivatives have showed good inhibitory effects against the fungi when the seeds were coated by these azo dyes [9]. Herein, three azo cresols consisting of anthracene moiety have been synthesized and subjected to an antifungal test against *A. niger***.**

2. Materials and Methods

2.1. Materials

Nitric acid, hydrochloric acid, anthracene, sodium hydroxide, phenol, were purchased from Carlo erba. Glacial acetic acid, sodium nitrite, sulfuric acid, *m*-cresol and *p*-cresol were purchased from Avonchem. Tin (II) chloride and *o*-cresol were purchased from PSPARK. Dimethylsulphoxide (DMSO) was purchased from BDH Chemical. All chemicals were used without further purification, Czapek Dox Agar was purchased from Oxoid LTD. Basingstoke, Hampshire, England. *Aspergillus niger* was isolated at Fungi Research Center, Assiut University, Egypt.

2.2. Instrumentation

Melting points were measured on a Barnstead electrothermal IA 9100. UV-Vis absorptions were recorded on UV-Vis spectrophotometer-uv mini 1240-Shimadzu. pH was measured using Jenway pH meter 3505. ^{1}HNMR spectra were recorded on a Bruker Avance 300 spectrometer. Residual proton signal from the deuteriated solvents were used as references [DMSO-d^6 (^{1}H, 2.50ppm, ^{13}C, 39.51 ppm) and $CDCl_3$ (^{1}H, 7.24 ppm, ^{13}C, 77.23ppm)]. Coupling constants were measured in Hz. Infrared spectra were recorded on Jasco FT/IR-4100 Fourier transform infrared spectrometer. Mass spectra were recorded on a Micromass Autospec M spectrometer. The electric measurements were carried out using laboratory power supply EA-PS 2016-050. The electrical current was measured in μA units by the use of CEM/DT- 3900 ammeter. The applied voltage was determined in V units by employing Peak Teck 2010 DMM voltmeter. Antifungal investigations were conducted using petri dishes 9 cm.

2.3. Preparation of 9-nitroanthracene (2) [10]

Concentrated nitric acid (4 cm^3) was added drop wise to a suspension of anthracene **1** (10.00 g, 56.00 mmol) in glacial acetic acid (40 cm^3) maintaining the temperature below 30 ºC. This was stirred vigorously for 1 h to form a clear solution. A mixture of concentrated HCl (50 cm^3) and glacial acetic acid (50 cm^3) was added slowly to the reaction vessel resulting to a pale yellow precipitate of 9-nitro-10-chloro-9,10-dihydroanthracene. This was filtered, washed with glacial acetic acid (3 × 25 cm^3) and thoroughly with water until the washings were neutral. The resulting yellow solid was treated with warm solution (60 – 70 ºC) of 10% NaOH (200 cm^3), filtered, washed with warm water until the washings were neutral, air-dried and recrystallized from glacial acetic acid affording a fluffy yellow solid (7.94 g, 35.60 mmol, 64% yield); m.p 156 ºC (lit[10] 153−157 ºC, acetic acid);

λ_{max} 448 nm ($CHCl_3$); FT-IR (KBr disc): 1511 cm^{-1} (C=C, aromatic), 1428 cm^{-1} (Ar-NO_2), 1278 cm^{-1} (C-N, aryl), δ_H (400 MHz; DMSO-d^6) 8.46 (1H, s, Ar-C*H*), 7.91 (2H, d, *J* = 8.70, Ar-C*H*), 7.83 (2H, d, *J* = 8.70, Ar-C*H*), 7.52 (2H, t, *J* = 15.11, Ar-C*H*), 7.44 (2H, t, *J* = 15.11, Ar-C*H*); δ_C (100 MHz; DMSO) 144.00 (1×Ar-*C*-NO_2), 134.00 (1×Ar-*C*), 130.41 (2 × Ar-*C*), 128.89 (2 × Ar-*C*) 127.92 (2 × Ar-*C*), 126.20 (2 × Ar-*C*), 122.64 (2 × Ar-*C*), 121.37 (2 × Ar-*C*); m/z ($C_{14}H_9NO_2$, Mwt. 223.23) 223.05 (43%), 197.88 (10%).

2.4. Preparation of 9-aminoanthracene (**3)** [10]

A suspension of 9-nitroanthracene **2** (7.24 g, 32.50 mmol) in glacial acetic acid (145 cm^3) was heated to 70 – 80 °C for 1½ h. To the resulting clear solution was added slurry of $SnCl_2$ (31.00 g, 163.20 mmol) in concentrated HCl (110 cm^3) via dropping funnel. The resulting yellow precipitate was stirred at 80 ºC for a further ½ h, cooled to room temperature, filtered, washed with concentrated HCl (3×10 cm^3), treated with solution of 5% NaOH while manual stirring from time to time for 15 min, filtered, washed thoroughly with water until the washing were neutral and vacuum-dried at 50 ºC for 6 h to afford a yellow powder (4.90 g, 25.39 mmol, 87% yield). No further purification was required; m.p 160 ºC (lit[10] 165 −170 ºC); λ_{max} 420 nm ($CHCl_3$); FT-IR (KBr disc): 3463 cm^{-1} (N-H), 3412 cm^{-1} (N-H), 1588 cm^{-1} (C=C, aromatic), δ_H (400 MHz; $CDCl_3$) 8.55 (1H, s, Ar-C*H*), 8.29 (2H, m, Ar-C*H*), 7.99 –7.28 (4H, m, Ar-C*H*), 6.80 – 6.72 (2H, m, Ar-C*H*), 4.88 (2H, br s , Ar-N*H*$_2$); δ_C (100 MHz; DMSO-d^6) 134.18 (1×Ar-*C*-NH_2), 132.21 (2 × Ar-*C*), 129.00 (2 × Ar-*C*), 128.52 (2 × Ar-*C*), 127.27 (2 × Ar-*C*), 125.27 (2 × Ar-*C*), 123.85 (2 × Ar-*C*), 121.11 (1×Ar-*C*); m/z ($C_{14}H_{11}N$, Mwt. 193.24) 193.30 (100%), 192.23 (25%), 194.28 (20%).

2.5. Preparation of 4-(9-anthrylazo)-2-methylphenol (4a)

An adapted literature procedure [10] was followed for synthesizing compound **4a**. 9-aminoanthracene **3** (0.77 g, 4.00 mmol) was dissolved in concentrated sulfuric acid (20 cm^3). The resulting solution was cooled to 0–5 ºC, to which a precooled (0–5 ºC) aqueous solution of sodium nitrite (0.55 g, 8.00 mmol; in 30 cm^3 water) was added while stirring for 30 min maintaining the temperature between 0 – 5 ºC. A solution of the *o*-cresol [0.43 g, 4.00 mmol; in 30 cm^3 of (2N NaOH and 2.5 g sodium carbonate)] was cooled to 0–5 ºC and subsequently added to the reaction mixture. The resulting mixture was stirred for further 1½ h at 0 – 5 ºC. A bright brown precipitate was formed, filtered, washed with cold water (3×10 cm^3) and air dried. The crude material was recrystallized from methanol, filtered, washed

with water (3×10 cm^3) and air dried to afford a mixture of two azo tautomers **4a** and **4b** (0.44 g, 1.41 mmol, 35 % yield) as a fine yellow powder. m.p 281 ºC; λ_{max} 421 nm ($CHCl_3$; FT-IR (KBr disc): 3442 cm^{-1} (Ar-OH), 1566 cm^{-1} (N=N), 1314 cm^{-1} (CH_3), 1277 cm^{-1} (C-N); δ_H (400 MHz; DMSO-d^6) 8.04 – 8.00 (1H, m, Ar-C*H*), 7.79 – 7.77 (1H, m, Ar-C*H*), 7.75 – 7.72 (2H, m, Ar-C*H*), 7.66 – 7.41 (4H, m, Ar-C*H*), 7.31 – 7.23 (2H, m, Ar-C*H*), 7.10 – 7.05 (2H, m, Ar-C*H*), 5.01 (1H, s, Ar-O*H*), 2.10 (3H, s, C*H*$_3$); δ_C (100 MHz; DMSO) 183.91 (1 × Ar-*C*-OH), 141.09 (1 × Ar-*C*), 135.70 (2 × Ar-*C*-N=N), 134.35 (2 × Ar-*C*), 133.47 (2 × Ar-*C*), 130.43 (2 × Ar-*C*), 130.06 (1 × Ar-*C*), 129.12 (1 × Ar-*C*), 128.07 (4 × Ar-C), 127.56 (2 × Ar-*C*), 126.89 (2 × Ar-*C*), 53.87 (1 × *C*H_3); m/z ($C_{21}H_{16}N_2O$, Mwt. 312.36) 312.03 (40%). Selected peaks for **4b**, δ_H (400 MHz; DMSO-d^6) 6.93 (1H, d, *J* = 9.00, C*H*) 6.60 (1H, d, *J* = 6.90, C*H*), 6.32 (1H, C*H*).

2.6. Preparation of 4-(9-anthrylazo)-3-methylphenol (5)

A modified literature procedure [10] was followed for the synthesis of compound **5**. 9-aminoanthracene **3** (0.77 g, 4.00 mmol) was dissolved in concentrated sulfuric acid (20 cm^3) and the resulting solution was cooled to 0–5 ºC, precooled (0–5 ºC) aqueous solution of sodium nitrite (0.55 g, 8.00 mmol; in 30 cm^3 water) was added to the reaction mixture while stirring for 30 min maintaining the temperature between 0–5 ºC. An alkaline solution of the *m*-cresol [0.43 g, 4.00 mmol; in 30 cm^3 of (2N NaOH and 2.5 g sodium carbonate)] was cooled to 0–5 ºC which was subsequently added to the reaction mixture. The reaction mixture was stirred for further 1½ h at 0–5 ºC. A brown precipitate was formed, filtered, washed with cold water (3×10 cm^3) and air dried. The crude material was recrystallized from glacial acetic acid, filtered, washed with water (3×10 cm^3) and air dried affording the desired azo dye **5** (0.5 g, 1.6 mmol, 40% yield) as a fluffy brown solid. m.p 290 ºC; λ_{max} 419 nm ($CHCl_3$); FT-IR (KBr disc): 3442 cm^{-1} (Ar-OH), 1670 cm^{-1} (N=N), 1324 cm^{-1} (CH_3), 1283 cm^{-1} (C-N); δ_H (400 MHz; DMSO-d^6) 7.76 – 7.70 (2H, m, Ar-C*H*), 7.54 – 7.42 (4H, m, Ar-C*H*), 7.25 – 7.21 (2H, m, Ar-C*H*), 7.13 – 7.09 (2H, m, Ar-C*H*), 7.00 – 6.96 (2H, m, Ar-C*H*), 5.02 (1H, s, Ar-O*H*), 1.91 (3H, s, C*H*$_3$); δ_C (100 MHz; DMSO-d^6) 183.79 (1 × Ar-*C*-OH), 149.19 (1 × Ar-*C*), 145.26 (2 × Ar-*C*-N=N), 144.69 (2 × Ar-*C*), 138.80 (2 × Ar-*C*), 135.85 (4 × Ar-*C*), 134.31 (2 × Ar-*C*), 128.06 (4 × Ar-*C*), 123.56 (2 × Ar-*C*), 60.71 (1 × *C*H_3); m/z ($C_{21}H_{16}N_2O$, Mwt. 312.36) 311.98 (100%), 297.61 (40%), 284.70 (37%).

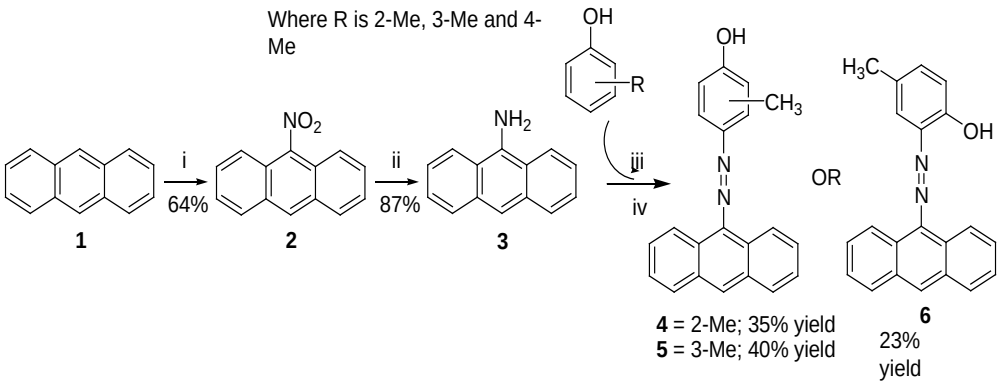
2.7. Preparation of 2-(9-anthrylazo)-4-methylphenol (6)

An adapted literature procedure [10] was followed for synthesizing compound **6**. 9-aminoanthracene **3** (0.77 g, 4.00 mmol) was dissolved in

concentrated sulfuric acid (20 cm^3). The resulting acidic solution was cooled to 0 –5 ºC, to which a precooled (0–5 ºC) aqueous solution of sodium nitrite (0.55 g, 8.00 mmol; in 30 cm^3 water) was added while stirring for 30 min maintaining the temperature between 0–5 ºC. An alkaline solution of the *p*-cresol [0.43 g, 4.00 mmol; in 30 cm^3 of (2N NaOH and 2.5 g sodium carbonate)] was cooled to 0–5 ºC and then added to the reaction mixture. The reaction mixture was stirred for further 1½ h at 0–5 ºC. A black solid substance was formed, filtered, washed with cold water (3×10 cm^3) and air dried. The resulting precipitate was recrystallized from glacial acetic acid, filtered, washed with water (3×10 cm^3) and air dried to give the desired azo dye **6** (0.29 g, 0.93 mmol, 23% yield) as a fluffy black solid. m.p 287 ºC; λ_{max} 410 nm ($CHCl_3$); FT-IR (KBr disc): 3437 cm^{-1} (Ar-OH), 1680 cm^{-1} (N=N), 1329 cm^{-1} (CH_3), 1283 cm^{-1} (C-N); δ_H (400 MHz; DMSO-d^6) 7.76 – 7.70 (2H, m, Ar-C*H*), 7.51 – 7.41 (4H, m, Ar-C*H*), 7.25 – 7.19 (2H, m, Ar-C*H*), 7.08 – 6.99 (2H, m, Ar-C*H*), 6.85 – 6.80 (2H, m, Ar-C*H*), 5.02 (1H, s, Ar-O*H*), 2.07 (3H, s, CH_3); m/z ($C_{21}H_{16}N_2O$, Mwt. 312.36) 312.09 (35%), 298.04 (15%), 284.84 (15%).

3. Results and Discussion

A nitration reaction of the anthracene **1** led to the formation of the 9-nitroanthracene **2** in a good yield (64%). A subsequent reduction process of the resulting 9-nitroanthracene 2 was carried out forming the corresponding 9-aminoanthracene **3** in an excellent yield (87%). The 9-aminoanthracene **3** was converted into a 9-anthryl diazonium bisulphate which was then coupled with cresol derivatives affording the desired azo dyes **4**–**6** in moderate yields (Scheme 1).



Reagents and conditions: (i) conc. HNO_3, HOAc, <30 °C, conc. HCl/HOAc, stirring, 1 h; (ii) slurry of $SnCl_2$ in conc. HCl, HOAc, stirring, 70 - 80 °C, 1.5 h; (iii) aq. $NaNO_2$, conc.H_2SO_4, 0 - 5 °C, 30 min; (iv) stirring, 0 - 5 °C, 1.5 h

Scheme (1): Synthesis of azo cresols

The resulting azo dyes **4−6** were characterized using a number of spectroscopic techniques e.g. uv-vis, IR, NMR and ms. The spectroscopic data revealed that only compound **4** exists in two tautomeric forms (azo/hydrazone tautomers) in ratio of (7:1), in which mixture of the two tautomers consists of 88% of the overall yield for the azo tautomer and just 12% for the other tautomer. However the other two azo compounds **5** and **6** have shown no sign of tautomerism [11].

3.1. Antimicrobial screening

Fungi are microorganisms, and most of them cause several types of diseases to humans and animals such as superficial mycoses, cutaneous mycoses, subcutaneous mycoses and systemic mycoses. Moreover, fungi attack plants and affect crops. *Aspergillus* is considered as one of the harmful fungi. Various species of this fungi are known to cause diseases in human which includes *A. fumigates, A. flavus, A. niger*.

3.2. The use of azo derivatives inhibiting agent as suspensions in water

The synthesized azo derivatives **4−6** were loaded on petri dishes containing Czapek Dox Agar media using a concentration of 0.025 g/ml as a suspension in distilled water, in form of three replicates for each azo derivative as well as Nystatin and Griseovin as references. In addition, three replicates of the control sample (neat Czapek Dox Agar media) were considered. Having placed a disk of the A.niger (with a diameter of 8 mm) in the middle of every petri dish, the samples were monitored every 24 hrs and the diameter of the fungus growth was measured in order to determine the inhibition percentage by applying the following equation [12]:

$$\text{Inhibition \%} = \frac{(\text{average of growth diameter for control} - \text{average of growth diameter for sample}) \times 100}{\text{average of growth diameter for control}}$$

The percentage of inhibition for all azo derivatives along with these for the references Nestatin and Griseovin showed low to moderate levels of inhibitory effects for the synthesized azo derivatives compared with Nystatin. However, some azo derivatives displayed as about equal inhibitory effects as that for the Griseovine.

Table (1): Inhibition percentage of fungi's growth

Compounds	Inhibition %
4	48
5	45
6	43
Nystatin (reference 1)	95
Griseovin (reference 2)	52

In addition, the azo derivatives of o-, *m*- and *p*-cresols **4–6** showed rather moderate inhibitory effects ranging from 43 to 48 % against the *A.niger*. The phenolic hydroxyl group in these compounds could be responsible for some kind of interaction between the azo derivative and the active sites of the fungi's enzymatic system which might lead to a distortion of the fungi's system in which the growth "replication" of the fungi is inhibited. This finding is in accordance with those in the literature where the phenolic compounds have been found to have inhibitory effects against wide variety of fungi [13, 14]. Moreover, plants have also been reported to produce phenolic compounds as a defence system against fungi [15]. In regard to the azo cresol derivatives **4–6**, the rather moderate inhibitory effects could be caused by the presence of the electron-donating methyl group along with the hydroxyl group on the benzene ring of the azo derivative. This helps the compounds **5** and **6** existing as phenolic azo forms rather than the corresponding hydrazone forms **5b** and **6b**, despite the fact that a tautomerism was observed in the case of *o*-cresol azo derivative **4**. It is only a small amount 12% of the hydrazone tautomer was formed Figure (1).

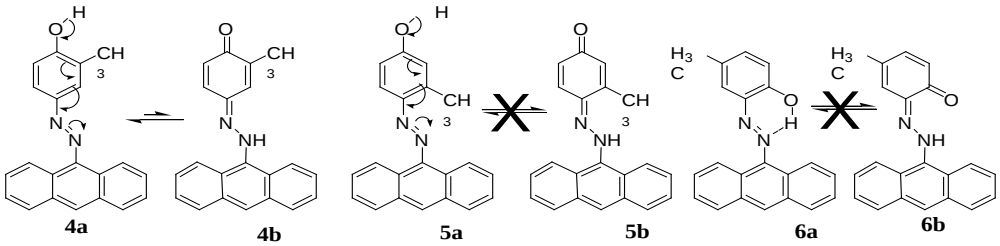


Figure (1): Tautomerism is allowed in 4 but disallowed in 5 & 6

The presence of methyl group on the benzene ring along with the hydroxyl group, assists pushing electrons towards the ring without the need most time for the hydroxyl group to play this role by which the tautomerism that leads to the formation of the hydrazo ketone (the disappearance of the phenolic OH) is disabled. This might allow the interaction between the phenolic hydroxyl group and the fungi to take place leading to inhibition of the fungi growth. Finally, all the synthesized compounds in this study showed much lower antifungal activities than the Nystatin. However they showed competitive inhibitory effects in comparison with the other reference in this study which is the Griseovine Table (1).

3.3. The use of azo derivatives inhibiting agent as solutions in DMSO

The antifungal activities of the synthesized compounds were tested against *A. niger* as a solution with concentration of 0.025 g/ml in DMSO on Czapek Dox Agar plate and the results were summarized in Table (2).

Table (2): Inhibition percentage of fungi's growth

Compounds	Inhibition
4	69
5	75
6	67

It could be clearly seen that, there is a notable increase in the levels of inhibition for all azo cresol derivatives **4–6** which showed good inhibitory effects ranging from 67% to 75% compared with using them with the same concentrations as suspensions in water.

4. Conclusion

Three azo cresols were synthesized in moderate yields, characterized and biologically tested against *A. niger*. The chemical structures of the three azo derivatives were proven using the available spectroscopic techniques e.g. uv-vis, IR, NMR and mass spectroscopy. The synthesized azo derivatives showed relatively moderate inhibitory effects against the *A niger* ranging from 43% to 48%, when they tested as suspensions in water. However, their inhibitory effects improved notably to be in the range of 67% to 75% once they were dissolved in DMSO and used as solutions.

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Nanoparticles as Antituberculosis Drugs: A review

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Abstract

The aim of the present review demonstrates the translational potential of nanotechnology to address the various challenges associated with the management of tuberculosis and improve current therapeutic strategies. Nanoparticles as a diagnostic tool include highly sensitive nanoparticle test which can potentially address many of the challenges outlined by the World Health Organization for the delivery of rapid and effective point of care diagnostics. The search for pioneering therapeutic approaches based on the use of new chemical compounds is gaining immense attention in modern therapy because of the increasing drug resistance. Tuberculosis (TB) in humans has been described since ancient times and its causative agent, *Mycobacterium tuberculosis* (MTB) is widely disseminated. The WHO estimates that approximately one-third of the global community is infected with M. tuberculosis. Approximately one-third of the world population is infected with *Mycobacterium tuberculosis*, resulting in more than eight million new cases and two million deaths annually. Recent implementation of the World Health Organization's strategy has been problematic, and TB remains a major burden in many developing countries. Despite mass *Mycobacterium bovis* BCG vaccination and the development of anti-tubercular drugs, tuberculosis still remains a major global public health problem. However nanotechnology has provided a huge improvement to pharmacology through the designing of drug delivery systems able to target phagocytic cells infected by intracellular pathogens, such as mycobacteria. The increased therapeutic index of anti-mycobacterial drugs; the reduction of dosing frequency; and the improvement of solubility of hydrophobic agents, allowing the administration of higher doses, have been demonstrated in experimental infections. Future research needs to include more comprehensive characterization, quality control and identification of antitubercular of clinical exposure with regards to composition and threshold. This review will shower the emerging vistas taken in this surge.

Keywords: *Mycobacterium tuberculosis*; Antitubercular; Nanotechnology; drug resistance.

1. Introduction

Tuberculosis (TB) in humans has been described since ancient times and its causative agent, *Mycobacterium tuberculosis* (MTB) is widely disseminated. The WHO estimates that approximately one-third of the global community is infected with M. tuberculosis [1]. The majority of TB cases are due to pulmonary tuberculosis (PTB). The main management approach is to eliminate the pathogen at its source. Thus, an understanding and good knowledge of PTB will be essential for controlling the spread and dissemination of TB. In 2009, an estimated 9.2 million incident cases and

approximately 1.7 million deaths due to TB occurred worldwide making it the worlds leading causes of mortality [2]. Approximately one-third of the world population is infected with *Mycobacterium tuberculosis*, resulting in more than eight million new cases and two million deaths annually. Although potentially curative treatments have been available for almost half a century, TB remains the leading cause of preventable deaths in the world today. Recent implementation of the World Health Organization's strategy (directly observed therapy, short-course) has been problematic, and TB remains a major burden in many developing countries. Regardless of mass *Mycobacterium bovis* BCG vaccination and the development of anti-tubercular drugs, tuberculosis still remains a major global public health problem. The clinical management of tuberculosis and other mycobacterial diseases with anti-mycobacterial chemotherapy remains a difficult task. The conventional treatment protocols are long-lasting; the drugs reach mycobacteria infected macrophages in low amounts and/or do not persist long enough to develop the desired anti-mycobacterial effect; and the available agents induce severe toxic effects. One of the major problems is noncompliance to prescribed regimens, primarily because treatment of TB involves continuous, frequent multiple-drug dosing. Adherence to treatment and the outcome of therapy could be improved with the introduction of long-duration drug formulations releasing the antimicrobial agents in a slow and sustained manner, which would allow reduction in frequency and dosing numbers. Nanotechnology has provided a huge improvement to pharmacology through the designing of drug delivery systems able to target phagocytic cells infected by intracellular pathogens, such as mycobacteria. The enlarged therapeutic index of anti-mycobacterial drugs; the reduction of dosing frequency; and the improvement of solubility of hydrophobic agents, allowing the administration of higher doses, have been demonstrated in experimental infections.

These recompenses may lead to new therapeutic protocols that will improve patient compliance and, consequently, lead to a more successful control of mycobacterial infections. One way to solve this problem is the development of colloidal drug delivery systems. Liposomes are a well-known example of this strategy [1, 2]. Other drug carriers (such as nanoparticles) represent an attractive alternative to liposomes. Today, versatility of particulate technologies enables tailoring of the nanoparticle-based drug delivery systems with consideration of the target, desired pharmacokinetic profile, and route of administration, The achievements and challenges of drug delivery using nanoparticles have been covered in

numerous publications over the last few years. The goal of the present review is to highlight the potential advantages of this research strategy relevant to the treatment of TB. Application of nanoparticles for the development of vaccines is beyond the scope of this article. Preliminary data by this group of authors on nanoparticles for TB drugs were published in 2000 [2].

2. Evolution of nanotechnology as diagnostic tool for tuberculosis

The diagnosis tools are required to meet the needs of the WHO's expansion of the Directly Observed Treatment Short-course, MDR and co-infection with HIV. In India, the country with the highest estimated number of TB cases, research is underway into the role nanotechnology can play in addressing such concerns. The Central Scientific Instruments Organization of India designed a nanotechnology-based TB diagnostic kit, which is currently in the clinical trials phase. This kit does not require skilled technicians for use and offers portability, efficiency, user-friendliness and availability for less than US$1. The research is also ongoing for an optical biosensor for rapid TB detection in the Medical Sciences division of the U.S. Department of Energy [3].

2.1. Nanoparticulate drug delivery system for tuberculosis treatment

The role of nanoparticles in the development of drug delivery systems is well established. In comparison to microparticles nanoparticles achieve a higher drug encapsulation and loading that result in enhanced bioavailability of encapsulated drugs. They dissolve rapidly in the gastrointestinal tract which can increase drug uptake as the local concentration of drug may be higher than conventional dosage forms. Further, in contrast to microparticles, nanoparticles cross the intestinal permeability barrier directly via transcellular/paracellular pathways which help for better delivery of the encapsulated drugs into the circulation. Nanoparticles for the purpose of drug delivery are defined as submicron (<1μm) colloidal particles. This definition includes monolithic nanoparticles (nanospheres) in which the drug is adsorbed, dissolved, or dispersed throughout the matrix and nanocapsules in which the drug is confined to an aqueous or oily core surrounded by a shell-like wall [3]. Alternatively, the drug can be covalently attached to the surface or into the matrix. Nanoparticles are made from biocompatible and biodegradable materials such as polymers, either natural (e.g., gelatin, albumin) or synthetic (e.g., polylactides, polyalkylcyanoacrylates), or solid lipids. In the body, the drug loaded in

nanoparticles is usually released from the matrix by diffusion, swelling, erosion, or degradation. The following are among the important technological advantages of nanoparticles as drug carriers: high stability (i.e., long shelf life); high carrier capacity (i.e., many drug molecules can be incorporated in the particle matrix); feasibility of incorporation of both hydrophilic and hydrophobic substances; and feasibility of variable routes of administration, including oral administration and inhalation. These carriers can also be designed to enable controlled (sustained) drug release from the matrix. The methods for nanoparticle preparation and characterization were addressed in numerous reviews, some of which are referenced here [4–8]. Table 1, summarizes major data on nanoparticulate formulations of the anti-TB drugs.

Table (1): Drug release and therapeutic efficacy of the nanoparticle-based formulations of the first-line anti-tubercular drugs

Delivery system	Animal model	Route of administration	Release duration		Regimen producing sterilizing effect in lungs and spleen	Ref
			Plasma	Organ		
PLG-NP	Mice	Oral	6-9	9-11	5 doses / 10 days	7
	Mice	Subcutaneous	32	36	Single injection	8
Lectin-NP	Guinea pigs	Aerosol	4-9	Up to 10 days	5 doses / 10 days	9
	Guinea pigs	Oral	4-9	Upto 10 days	5 doses / 10 days	9
	Guinea pigs	Oral	7–13	Up to 15 days	3 doses / 15 days	9
	Guinea pigs	Aerosol	6–14	Up to 15 days	3 doses / 15 days	9
Solid lipid nanoparticles (SLN)	Guinea pigs	Aerosol	5	7	7 doses / week	10

2.2. Anti-tubercular nanoparticles for oral administration

Immovability of nanoparticles suggestions the possibility of oral administration. The fate of nanoparticles in the gastrointestinal tract has been investigated in a number of studies [9–11]. In general, the uptake of nanoparticles occurs as follows: [1] by transcytosis via M cells, [2] by intracellular uptake and transport via the epithelial cells lining the intestinal mucosa, [3] by uptake via Peyer's patches.

Pandey et al. revealed that the nanoparticles provided sustained release of the anti-TB drugs and considerably enhanced their efficacy after oral administration [12]. Three frontline drugs, rifampin (RMP), isoniazid (INH), and pyrazinamide (PZA) were coencapsulated in poly (lactide-co-glycolide) (PLG) nanoparticles. After a single oral administration of this formulation to mice, the drugs could be detected in the circulation for 4 d (RMP) and 9 d

(INH and PZA); therapeutic concentrations in the tissues were maintained for 9 to 11 d. In contrast, free (unbound) drugs were cleared from the plasma within 12 to 24 h after administration. Treatment of *M. tuberculosis*–infected mice with the nanoparticle-bound drugs (five oral doses every 10th day) resulted in complete bacterial clearance from the organs. Free drugs were able to produce bacterial clearance only after daily administration of 46 doses. Similar efficacy of the nanoparticle-bound drugs was also observed in guinea pigs [13]. Simultaneously, incorporation in microparticles was less effective: their drug-loading capacity was lower as well as the plasma half-life of the bound drugs [14, 15].

The behavior of polymeric nanoparticles in the gastrointestinal tract is influenced by their bioadhesive properties; adhesion of nanoparticles to the mucosa enhances the absorption of the associated drug, thus increasing its bioavailability. Thus, lectins have been shown to improve mucoadhesion of the drug due to the biorecognition of the lectin-grafted carriers by glycosylated structures in the intestine [16].

Consequently, the efficacy of PLG-based formulations of anti-TB drugs was further improved by covalent attachment of wheat germ agglutinin [17]. Oral administration of wheat germ agglutinin–coated PLG nanoparticles loaded with RIF, INH, and PZA in mice produced considerably extended serum half-life: detectable RIF serum levels were observed for 6 to 7 d and INH and PZA for 13 to 14 d (vs. 4–6 d and 8–9 d for nonmodified nanoparticles). All three drugs were present in lungs, liver, and spleen for 15 d. The lectin-modified formulations produced bacterial clearance in these organs after three oral doses administered every 14 d (vs. 45 daily doses of free drugs). As suggested by the authors, the prolonged circulation of drugs encapsulated in wheat germ agglutinin–grafted nanoparticles might be attributed to the fact that lectins enhance prolonged adhesion of the particles to the intestinal surface to allow [1] an increase in the time interval available for absorption and [2] a localized increase in the concentration gradient between luminal and serosal sides of the membrane.

2.3. Anti-tubercular nanoparticles for inhalation

The potential advantages of direct delivery of the TB drug to the lungs include the possibility of reduced systemic toxicity, as well as achieving higher drug concentration at the main site of infection. Moreover, in contrast to the oral route of administration, inhaled drugs are not subjected to first-pass metabolism. A possible obstacle to using nanocarriers for pulmonary

delivery is that their mass median aerodynamic diameter, an essential parameter for the particle deposition in the lungs, is often too small.

However, the effectiveness of pulmonary drug delivery using nanoparticles was demonstrated in a number of studies [18]. The pharmacokinetics and antibacterial effect of the nanoparticle-bound anti-TB drugs administered via respiratory route was investigated in guinea pigs [19]. The dose was delivered via a suitable facemask connected to the compressor–nebulizer system. A single nebulization of RMP, INH, and PZA coencapsulated in PLG nanoparticles to guinea pigs resulted in sustained therapeutic drug levels in the plasma for 6 to 8 d and in the lungs for up to 11 d. This effect was similar to that obtained after oral administration of the nanoparticulate formulation of the same drugs. In nebulization of nanoparticles to *M. tuberculosis*–infected guinea pigs at every 10th day, no tubercle bacilli could be detected in the lung after only five doses of treatment, whereas 46 daily doses of orally administered drug were required to obtain an equivalent therapeutic benefit.

Administration to infected guinea pigs of nebulized RMP, INH, and PZA coencapsulated in wheat germ agglutinin–functionalized PLG nanoparticles was even more effective: three doses administered fortnightly for 45 d were sufficient to produce a sterilizing effect in lungs and spleen [17]. A sanitizing effect was also achieved when the drugs were loaded in solid lipid nanoparticles [20]. No tubercle bacilli could be detected in the lungs/spleen after seven doses of treatment of infected guinea pigs with drug-loaded solid lipid nanoparticles. It is noteworthy that the solid lipid nanoparticles display important advantages, such as the composition (physiologic compounds) and the possibility of large-scale production favored by the feasibility to avoid organic solvents in the manufacturing process [10].

2.4. Anti-tubercular nanoparticles for Intravenous administration

In divergence to microparticles with a diameter of more than 1 μm that cannot be administered via intravascular routes, nanoparticles are small enough to allow intracapillary passage followed by an efficient cellular uptake. When administered intravenously, the nanoparticles follow the route of other foreign particulates, including intracellular pathogens. They are endocytosed by resident macrophages of the mononuclear phagocyte system and by circulating monocytes. On the other hand, in the case of infections caused by intracellularly persisting microbes (e.g., *Brucella, Salmonella,*

Listeria, Mycobacterium), macrophages become reservoirs for pathogens, thus representing one of the targets for delivery of antimicrobial agents.

Improved uptake of nanoparticles by macrophages (mainly by Kupffer cells in the liver) is achieved by the physicochemical properties of the carrier and by physiologic opportunity, thus representing an example of passive delivery. This technology improves drug delivery to macrophages, increasing the amount of the drug reaching this target site, which allows reduction of the overall therapeutic dose and decrease of the adverse effects. Accordingly, the enhanced efficacy of the nanoparticle-bound antibiotics was demonstrated in a number of experimental infections [21, 22].

The prospective of macrophage-targeting strategy in development of the nanoparticle-based TB drugs is supported by the *in vitro* data. Incorporation of INH and streptomycin in poly(butyl cyanoacrylate) nanoparticles not only increased the intracellular accumulation (or association) of these drugs in the cultivated human blood monocytes but also produced enhanced antimicrobial activity of these agents against intracellular *M. tuberculosis* compared with their activity in extracellular fluid [23]. Similarly, the encapsulated ciprofloxacin [24] and RMP [25] produced the enhanced effect against mycobacteria in the infected macrophages. This is in contrast to the previous *in vitro* observations showing that enhanced intracellular accumulation of drugs in macrophages is rarely associated with a simultaneous increase of activity against intracellular mycobacteria (compared with activity against the extracellular bacterial population). This is presumably due to the fact that the drugs and bacteria are sequestered in different intracellular compartments. It is noteworthy that the enhanced cell uptake and activity against intracellular bacteria was demonstrated for the nanoparticulate streptomycin, an aminoglycoside agent, which, in the free form, has poor intracellular access.

Clofazimine, a riminophenazine compound, is an agent considered for treating patients with *M. avium* infection. However, use of this drug was restricted because of its poor solubility. A relatively new approach was applied to solve the problem: clofazimine was formulated as a nanosuspension consisting only of the drug and a minimum amount of surfactants (particle size, 385 nm). Intravenous injection of the nanocrystalline formulation of clofazimine resulted in a considerable reduction of bacterial loads in the liver, spleen, and lungs of mice infected with *M. avium* [26]. This result correlated with the pharmacokinetic data:

drug concentrations in these organs reached high concentrations, well in excess of the minimal inhibitory concentration for most *M. avium* strains. Interestingly, the effects of the nanocrystalline formulation of clofazimine were similar to those of the liposomal formulation used as a control in this study. This study is a vivid example of application of nanotechnology for overcoming the solubility problems of poorly soluble drugs. More details on the application of nanosuspensions in drug delivery can be found in a review on this subject [27]. Intravenous administration of the nanoparticles has the further advantage of passive drug delivery to inflammatory sites where the endothelium becomes permeable due to pathologic processes. In this case, passive delivery of the nanoparticles would be realized by pathophysiologic opportunity. The basic principles for engineering of the nanocarriers suggest that passive accumulation in the sites with leaky vasculature would be more effective with the long-circulating (stealth) nanoparticles [28]. These particles evade resident macrophages in the liver and have higher probability to reach other sites in the body.

2.5. Anti-tubercular nanoparticles via other routes of administration

Flexibility of the nanoparticle-based formulations was further demonstrated by effective subcutaneous treatment of mice infected with *M. tuberculosis* [29]. A single subcutaneous dose of PLG nanoparticles loaded with RMP, INH, and PZA maintained therapeutic drug levels in plasma for 32 d and in lungs/spleen for 36 d. Moreover, this single subcutaneous injection produced a sterilizing effect in lungs and spleen of the infected mice (36 d post-treatment), thereby demonstrating a better chemotherapeutic efficacy, as compared with daily treatment using free drugs (35 oral doses). As suggested by the authors, the nanoparticles form a depot at the injection site that is slowly releasing drugs into the circulation.

2.6. Vaccination for tuberculosis via nanotechnology

The aerosol vaccine- under development through collaboration between Harvard University and the international not-for-profit Medicine in Need (MEND) - could provide a low-cost, needle-free TB treatment that is highly stable at room temperature. While most new TB vaccines continue to call for needle injection, but this new vaccine could provide safer, more consistent protection by eliminating these injections and the need for refrigerated storage.

A successful result of aerosol delivery using nanoparticle technology offers a potentially new policy for immunization. Among guinea pigs vaccinated with the aerosol treatment and subsequently exposed to TB, less

than 1 percent of lung and spleen tissue showed effects of the disease. By contrast, in animals treated with the same dose of the traditional injected vaccine, some 5 percent of lung tissue and 10 percent of spleen tissue showed symptoms following TB exposure. In the aerosol vaccine, particles form at micrometer and nanometer scales and in spherical and elongated shapes, a combination that appears to improve dispersal in the mouth. While commonly used with food, cosmetics, and pharmaceuticals, this spray drying of small and large molecules is seldom used for drying cellular material. The new technique enables TB vaccines, and potentially other bacterial and viral-based vaccines, to sidestep the traditional problems associated with keeping vaccines chilled. Furthermore, a nanotechnology-based vaccine adjuvant for TB was developed by the U.S firm, Biosante, in 2002 [3].

3. Conclusions

Scientific developments and increasing international attention have promoted our ability to work with and understand the nano scale. Nanotechnology provides a new focus for research through its aim to manufacture from the 'bottom-up' rather than from the 'top down'. It also demands an unprecedented collaborative and integrated approach to science and technology. In an area such as tuberculosis, nanotechnology has the potential to empower a local response to challenges such as the diagnosis and treatment and prevention of this deadly disease and we can see it's as a better approach to solve out the all problems.

Although identifying novel anti-TB agents remains a priority, the development of the nanoparticle-based delivery systems for currently used agents may represent a cost-effective and promising alternative. The above data suggest that nanoparticles have a considerable potential for treatment of TB. Their major advantages, such as improvement of drug bioavailability and reduction of the dosing frequency, may create a sound basis for better management of the disease, making directly observed treatment more practical and affordable. Another important advantage of the nanoparticles is the feasibility of the versatile routes of drug administration, including oral and inhalation routes. In addition, high stability of the nanoparticles suggests long shelf life.

It can be expected that future research will concentrate on the development of the vectorized delivery systems combining advantages of the colloidal carriers, such as large payloads of a drug, with active targeting

to the infection sites. Moreover, development of innovative formulation technologies suggests that nanoparticles can be incorporated into various solid dosage forms (microparticles, granules, or tablets), which can release the nanoparticles at the site of action, preserving their original properties [30–32]. These approaches would further improve efficacy and practicability of the nanoparticle-based formulations.

Finally, the success of this technology will probably depend on toxicologic issues associated with understanding of the fate of nanocarriers and their polymeric constituents in the body, as well as elimination of the risk of the residual organic solvents. In this respect, the possibility of using drug carriers made from natural polymers (e.g., chitosan or alginate) represents an attractive perspective.

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Extrahepatic Cholangiocarcinoma: Experience from Misurata Cancer Center, Libya (2012-2014)

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Abstract

Twenty five patientswere registered in Misurata Cancer Center between January, 2012 and June, 2014 to haveextrahepaticcholangiocarcinoma (ECC). The median age was 52 years (range from 32-78 years). Eighteen patients(72%) were males and7 patients (28%) were females.Fourteen patients had ECOG performance status score 0 or 1 and 11 patients had score 2 or 3. All the patients presented with obstructive jaundice. Fifteen patients (60%) had perihilar (Klatskin) tumors, and the remaining (40%) had distal bile duct tumors. Twelve patients had cytological or histological confirmatory diagnosis. Six patients underwent preoperative laparoscopic evaluation before surgery, 2 of them proved to have disseminated peritoneal deposits and rendered inoperable.Atotal 21 patient were rendered to have unresectable tumor due to either extensive liver infiltration (6 patients), ornon regional LN metastasis and/or distant metastasis (15patients).Four patients underwent Whipple surgery, one patient died due to sepsis and hemorrhage,and 3 patients remained alive till time of analysis, one of them received 6 cycles of adjuvant chemotherapy (gemcitabine and cisplatin) due to lymph node metastasis. Of the remaining 21 patients, 18 patients underwent biliary stenting guided by ERCP (16 patients) or PTC (2 patients). The bilirubin was normalized in 15 patients. A total of 13 patients received palliativechemotherapy, gemcitabine/cisplatin regimen. The total number of chemotherapy cycles was 67 cycles (range from 2-9 cycles per patient). Eight of 13 patients (61.5%) who received chemotherapy had any ≥ grade 3 (severe) toxicity. The most common severe chemotherapy toxicities were neutropenia (6 patients 46%), anemia (4 patients 31%), thrombocytopenia (3 patients 23%), neutropenic fever (2 patients 15%), nausea and vomiting (3 patients 23%), and lethargy(2 patients 15%).Complete response (CR) was achieved in 1 patient (7.6%), partial response (PR) in 3 patients (23%) and stable disease (SD) in 5 patients (38.4%) while 4 patients (31%) had disease progression (DP) during therapy.The median progression free survival (PFS) was 4.4 months. The median overall survival (OS) was 8.4 months.

Keywords*:* Cholangiocarcinoma, Gemcitabine, Cisplatin.

1. Introduction

Cholangiocarcinoma is a relatively rare neoplasmthat is classified as an adenocarcinoma.Cholangiocarcinoma can affect any area of the bile ducts, either within or outside the liver. Tumors occurring in the bile ducts within the liver are referred to as intrahepatic (ICC), those occurring in the ducts

outside the liver are extrahepatic (ECC), and tumors occurring at the site where the bile ducts exit the liver may be referred to as perihilar. A cholangiocarcinoma occurring at the junction where the left and right hepatic ducts meet to form the common hepatic duct may be referred to eponymously as a Klatskin tumorCholangiocarcinoma is considered to be an incurable and rapidly lethal malignancy [1]. No potentially curative treatment yet exists except surgery, but most patients have advanced stage disease at presentation and are inoperable at the time of diagnosiswith overall median duration of survival is less than 6 months. Patients with advanced cholangiocarcinoma are generally managed - though never cured - with chemotherapy, radiation therapy, or other palliative care measures [2]. During the past decade, many cytotoxic drugs with activity in advanced biliary tract cancer were identified, including 5-fluoro-uracil, gemcitabine, capecitabine, oxaliplatin, and irinotecan, tyrosine kinase inhibitors like erlotinib or sorafenib or monoclonal antibodies like bevacizumab or cetuximab [3,4]. Recently, based on the results of many randomized trials, the combination of gemcitabine and cisplatin is considered to be the standard of care as the first line therapy for patients with advanced or metastatic biliary tract cancer [5-9]. The aim of this retrospective study is to evaluate the outcome of extrahepatic cholangiocarcinoma patients

2. Patient and Methods

This is a retrospective study including patients registered at MCC between January, 2012 and June, 2014and diagnosed to have ECC.Prior to treatment, all patients should have adequate organ function as follows: (1) adequate bone marrow reserve (WBC count >4000/µl, platelet count >100 000/µl); (2) normal renal function (serum creatinineor creatinine clearance if abnormally elevated creatinine clearance); (3) adequate hepatic function [aspartate aminotransferase (AST) and alanine aminotransferase (ALT) <3 times the upper normal limit and total bilirubin <1.5 times the upper normal limit]; and (4) normal cardiac function.Additionally, the measurement of tumor markers CEA and CA19.9 was essential before and after the relief of the biliary obstruction. Radiologically, all the patients underwent chest X-ray, computerized tomography (CT) of chest and abdomen, magnetic resonant imaging (MRI) of the abdomen and magnetic resonant cholangiopancreatography (MRCP). All patients who suspected to have inoperable cholangiocarcinoma and had obstructive jaundice should do endoscopic retrograde cholangiopancreatography (ERCP) or percutanoustranshepatic cholangiography (PTC) for possible imaging, stenting and cytological or histopathological evaluation. All patients who

suspected to have operable cholagniocarcinoma underwent laparoscopic evaluation before surgery to exclude any subradiological metastasis. Patients with intrahepatic cholangiocarcinoma, gall bladder cancer and ampulla of Vater caner were not included. All patients and/or their legal representatives signed informed consent forms.

2.1. Treatment plan

The patients who were medically and surgically candidate forcomplete resection underwent Wipple surgery. The patients who had positive or close margins or positive lymph node metastasis received 4-6 cycles of adjuvant chemotherapy, gemcitabine and cisplatin after complete recovery from surgical morbidity. Inoperable patients with good performance status, after relieving the biliary obstruction through stenting, were candidates for palliative chemotherapy, gemcitabine and cisplatin, one cycle every 21 days. Treatment was discontinued because of disease progression, patient choice, or intolerable toxicity. Biliary obstruction during therapy per se was not considered to be disease progression in the absence of radiologically confirmed disease progression, and treatment could be recommenced after further biliary stenting and normalization of liver function. Patients who were clinically unfit for palliative chemotherapy or refused chemotherapy underwent best supportive care.

The chemotherapy regimen consisted of gemcitabine 1000 mg/m^2 intravenous (i.v.) on day 1 and day 8, andcisplatin 75 mg/m^2i.v. on day 1, given every 21 days.Gemcitabine /cisplatin regimenwas administered on an outpatient basis. Appropriate i.v. hydration was given along with the cisplatin(1 liter of 0.9% saline containing, 20 mmol of potassium chloride, and8 mmol of magnesium sulfate over 1-2 hour followedby 500 ml of 0.9% saline containing cisplatin over 60 minutes concomitant or followed by manitol 20%and diuretics.. All patientsreceived gemcitabine as a 30-minute infusion. Allpatients should receive adequate antiemetic measurements,infusion of 250 cc normal saline or 5% glucose over 30 minutes containing 5-HT3 antagonist (granisetrone or ondanosetrone), dexamethazone 8mg, and rantidinehydrocholoride 50 mg,before therapy.Dose modifications were defined per protocol, and modifications and delays were allowed for hematologic and non hematological toxicities.The dose of gemcitabine was adjusted on day 8 based on WBC, platelet count and liver function test. If the absolute neutrophil count (ANC) was <1000/µl or the platelet count was <75 000/µl on day 8, the dose of gemcitabine was reduced by 20%. If ANC was <500/µl or the platelet count was <50 000/µl, gemcitabine was omitted. If the serum bilirubin level was

2.0–3.0 mg/dl and AST/ALT was <5 times the upper normal limit, the dose of gemcitabine was reduced by 20%. If the serum bilirubin level was >3.0 mg/dl or AST/ALT was >5 times the upper normal limit, gemcitabine was also omitted. If ANC was <1500/µl or the platelet count was <75 000/µl at the start of a new cycle, treatment was delayed for recovery without dose reduction. The cisplatin dose was modified based on creatinine clearance level. If creatinine clearance was< 60 ml/min, carboplatin AUC 4 was substituted for cisplatin. If creatinine clearance was < 40 ml/min, the platinum drugs were omitted. If grade 3/4 hematological or non hematological toxicities developed following any dose reduction, treatment was stopped.

2.2. Toxicity

Patients were seen at the start of every cycle for a physical examination, monitoring of any new or progressive symptoms or signs, andmonitoring of renal, liver functions and blood count recovery.Before each cycle, patients were assessed for treatment toxicity according to National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 3.

2.3. Assessment of response

Tumor response was assessed subjectively and objectively byCEA and CA 19.9 measurement and by means of computed tomography (CT) or magnetic resonance imaging (MRI) that done every 2 chemotherapy cycles, at the end of treatment and then every 3 months for the first year then every 6 month on the second year.Response to treatment was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria[10]. Completer Response (CR) was defined asdisappearance of all target lesionsand normalization of tumor marker level. Alllymph nodes must be non-pathological in size(<10mm short axis). Partial Response (PR) was defined asat least a 30% decrease in the sum ofdiameters of target lesions, taking as reference thebaseline sum diameters. Disease Progression (DP) was defined as an appearance of new lesion (s) orat least a 20% increase in the sumof diameters of target lesions; taking as referencethe the baselinesum diameters. In additiontothe relative increase of 20%, the sum must also demonstratean absolute increase of at least 5 mm. Stable Disease (SD) was defined as neither PR nor DP criteria met.

2.4. Survival

Progression free survival (PFS) was defined from the first day of chemotherapy to the day of confirmed progression. Overall survival (OS)

was calculated from the first day of chemotherapy with gemcitabine and cisplatin to death or last follow-up.

3. Results and Discussion

3.1. Patient Characteristics

Twenty five patientswere registered in Misurata Cancer Center between January, 2012 and June, 2014 to have ECC. The median age was 52 years (range from 32-78 years). Eighteen patients (72%) were males and 7 patients (28%) were females.

Table (1): Patient characteristics

Age—yrs	
Median	52 yrs
Range	(32-78) yrs
Sex--- no. (%)	
Male	18(72%)
Female	7 (28%)
ECHO Performance status score --- no. (%)	
0	6 (24%)
1	8(32%)
2	6(24%)
3	5 (20%)
Predisposing factors--- no. (%)	
Chronic calcularcholecystitis	19(76%)
Previous cholecystectomy	10(40%)
HCV infection	6 (24%)
Presenting symptoms & signs--- no. (%)	
Obstructive Jaundice	25 (100%)
Abdominal pain	8(32%)
Anorexia	10 (40%)
Fever	2 (8%)
Extra-hepatic biliary duct site--- no. (%)	
Perihilar (Klatskin)	15(60%)
Distal CBD	10(40%)
Extent of disease--- no. (%)	
Localized	4 (16%)
Locally advanced	6 (24%)
Metastatic	15(60%)
Type of therapy--- no. (%)	
Surgery: Whipple operation	4 (16%)
Palliative Chemotherapy	13(52%)
Best supportive care	8 (32%)

Fourteen patients had ECOG performance status score 0 or 1 and 11 patients had score 2 or 3. Nineteen patients (76%) had a history of repeated attacks of biliary colic due to chronic calcularcholecystitis and 10 of them had previous cholecystectomy. Additionally 6 patients (24%) had been screened positive for HCV infection. All the patients presented with obstructive jaundice. In addition, 8 patients (32%) had right side abdominal pain, 10 patients (40%) were suffered from anorexia and,2 patients (8%) were feverish at presentation.Fifteen patients (60%) had perihilar (Klatskin) tumors, and the remaining (40%) had distal bile duct tumors. Twelve patients had cytological or histological confirmatory diagnosis. The remaining 13 patients had clinical and radiological diagnosis only, including patients who planned to have surgical intervention. Six patients underwent preoperative laparoscopic evaluation before surgery, 2 of them proved to have disseminated peritoneal deposits and rendered inoperable. Total 21 patient were rendered to have unresectable disease due to extensive liver infiltration (6 patients), ornon regional LN metastasis and/or distant metastasis (15 patients). Four patients underwent Whipple surgery, one patient died due to sepsis and hemorrhage, and 3 patients remained alive till time of analysis, 2 of them did not receive adjuvant treatment and one patient received 6 cycles of adjuvant chemotherapy (gemcitabine and cisplatin) due to lymph node metastasis. Of the remaining 21 patients, 18 patients underwent biliary stenting guided by ERCP (16 patients) or PTC (2 patients). The bilirubin was normalized in 15 patients. A total of 13 patients received palliative chemotherapy. Eight patients referred to palliative care unit either due to poor performance status (5 patients) or refusal of chemotherapy (3 patients). The details of patient characteristics are shown in Table (1).

3.2. Toxicity

Eight of 13 patients received palliative chemotherapy (61.5%) had any ≥ grade 3 (severe) toxicity. The most common severe chemotherapy toxicities were neutropenia (6 patients 46%), anemia (4 patients 31%), thrombocytopenia (3 patients 23%), neutropenic fever (2 patients 15%), nausea and vomiting (3 patients 23%), lethargy(2 patients 15%) and, renal impairment (2 patients 15%). Gemcitabine dose was reduced by 20% in 4 patients who developed ≥ grade 3 hematological toxicity. Carboplatin AUC 4 was substituted for cisplatin in one patient who had renal impairment with creatinine clearance > 40 ml/min and platinum drug was totally omitted in the other patient who had severe renal impairment with creatinine clearance

< 40 ng/ml. The detail of chemotherapy severe toxicity is shown in Table (2).

Table (2): Chemotherapy toxicity (≥grade 3 toxicity (NCI-CTC) version 3)

Type of toxicity	No. (%)
Any≥ grade 3 chemotherapy toxicity	8/13 (61.5%)
Hematological toxicity	
Neutropenia	6/13 (46%)
Anemia	4/13 (31%)
Thrombocytopenia	3/13 (23%)
Neutropenic fever	2/13 (15%)
Non-hematological toxicity	
Nausea & Vomiting	3/13 (23%)
Lethergy	2/13 (15%)
Renal impairment	2/13 (15%)

3.3. Response

The total number of palliative chemotherapy cycles given for the 13 patients was 67 cycles (range from 2-9 cycles per patient). As shown in Table (3), complete response (CR) was achieved in 1 patient (7.6%), partial response (PR) in 3 patients (23%) and stable disease (SD) in 5 patients (38.4%) i.e. objective response (CR+ PR+ SD) was recorded in 9 patients (69%). Four patients (31%) had disease progression (DP) during therapy.

Table (3): Response to treatment (according to RECIST criteria)

Variable	No. (%)
Complete Response (CR)	1/13 (7.6%)
Partial Response (PR)	3/13 (23%)
Stable Disease (SD)	5/13 (38.4%)
Progressive Disease (PD)	4/13 (31%)

3.4. Survival

The median progression free survival (PFS) for patients receiving palliative chemotherapy for locally advanced or metastatic disease was 4.4 months. The median overall survival (OS) was 8.4 months.

Cholangiocarcinoma is a relatively rare neoplasm that has an annual incidence rate of 1–2 cases per 100,000 in the Western world [11], but rates of cholangiocarcinoma have been rising worldwide over the past several decades[12]. The rate of ECC has been rising dramatically in Libya over the last few years. This might be attributed to more health orientation and education, to the high prevalence of chronic calcularcholecystitis, tothe higher incidence of hepatitis C (HCV) infection than before or alltogether. In MCC, 25 cases were registered within one and half year representing the

6th most common disease. The disease was more prevalent in males and in old age patients (5th -6thdecade). Forty percent of the patients had a history of cholecystectomy and 24% had HCV infection.Perihialr (Klatskin) tumors were more prevalent than other distal CBD tumors (60% vs. 40%).

Complete resection with negative margins is the only potential curative treatment for resectable cases. The reported 5 year survival rates following radical resection in the range of 20-42% and 16-52% respectively, for patients with hilar and distal cholangiocarcinoma [13]. Surgery is contraindicated in patients with distant metastatic disease to the liver, peritoneum or distant lymph nodes beyond portahepatis and in patients with co morbidities that interfere with this extensive surgical procedure [14]. Unfortunately, in the present study only, 4 patients (16%) were medically and surgically operable, one patient died due to sepsis and hemorrhage i.e 25% mortality rate, and 3 patients remained alive till time of analysis, one of them received 6 cycles of adjuvant chemotherapy due tolymph node metastasis. Due to the low incidence of biliary tract cancers, the efficacy and safety of adjuvant chemotherapy orchemo radiation have been evaluated in retrospective studies that have included only a small number of patients, these studies often combined patients with gall bladder cancer and bile duct cancers. These retrospective studies provided conflicting evidence regarding the role of adjuvant therapy.In a recent systematic review and meta-analysis of 6,712 patients with biliary tract cancers, Horgan et al reported an improvement in OS (although not significant) with the addition of adjuvant therapy with the greatest benefit observed in patient with macroscopic residual disease and lymph node metastasis [15].

The prognosis of the patients with advanced biliary cancer is very poor and the median survival for those undergoing supportive care alone is very short. The results of a pooled analysis of 104 trials that have included 2810 patients with advanced biliary tract cancers showed that the response rate and the tumor control were higher among the subgroup of patients receiving a combination of gemcitabine and platinum based agents [16]. In the present study, 21 patients (84%) have locally advancedor disseminated disease at presentation, and only 13 patients were candidates to receive palliative chemotherapy. Cisplatin/ gemcitabine combination were tolerated well by the patients although 61.5% of patients had severe toxicity during chemotherapy.The most common severe toxicities were neutropenia (46%), anemia (31%), thrombocytopenia (23%) and nausea and vomiting (23%). The objective response rate was recorded in 69% of cases. The median PFS and OS were 4.4 month and 8.4 months respectively.In the randomized,

controlled, phase III ABC-02 study, Valle *et al* [17] treated more than 400 patients with locally advanced biliary tract carcinomas with either gemcitabine (1000 mg/m^2)/ cisplatin (25 mg/m^2) regimen, each administered at days 1 and 8 every 3 weeks for 8 cyclesvs gemcitabine only.As compared with gemcitabine alone, cisplatin plus gemcitabine was associated with a significant survival advantage without the addition of substantial toxicity. Of 198 patients assessed for toxicity to gemcitabine / cisplatin regimen, 70.7% of patientshad severe toxicity during therapy. The most common severe toxicities wereneutropenia (25.3%), anemia (7.6%), thrombocytopenia (8.6%) and nausea and vomiting (9%). Of 161 patients assessed for response to gemcitabine / cisplatinregimen, 81.4% of the patients achieved objective response.The median PFS was 8.0 months and the median OS was 11.7 months.The better results in this multicenter trial compared to the present study may be attributed to the smaller number of the patients in the present study, and much less patients in the present study had ECOG 0 or 1 (56% vs.86.8%).

ECC is still a very fatal disease mainly because of late disease discovery and lack of effective chemotherapeutic agents.Some areas of ongoing medical research in cholangiocarcinoma includethe techniques to measure the concentration of byproducts of cancer stromal cell formation in the blood for diagnostic purposes and the use of newer targeted therapies, (such as erlotinib) or photodynamic therapy for treatment.

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Case Report
Diffuse Large B-cell Lymphoma with Clear Cells Morphology: A rare Variant

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Abstract

Diffuse large B-cell lymphoma is a rare type of non-Hodgkin lymphoma composed of diffuse proliferation of large neoplastic B lymphoid cells with a nuclear size equal to or exceeding the normal macrophage nuclei. It has become clear that diffuse large B-cell lymphoma is not a uniform category. Several recognizable variants have clinically distinct features and, frequently, require specific treatment approaches. Recognition of these variants and utilization of the appropriate treatments will improve the outcome for the patients. We report a case of a clear cell variant of diffuse large B-cell lymphoma involving a lymph node in the neck, which was clinically suspected of being metastatic carcinoma. A 50-year-old Libyan woman from Misurata presented with cervical lymphadenopathy and rapidly enlarging retrosternal mass which radiologically was 15.0 x 7.0 cm. Core biopsy of the cervical mass was performed at Misurata Cancer Center (MCC). The biopsy showed a malignant infiltration exhibiting diffuse areas comprising large cells with slightly irregular nuclei and clear cytoplasm admixed with a few mononuclear cells. In these areas, there was high mitotic activity, staining for cytokeratins was negative. These areas had the following phenotypes: cluster designation marker 20 (CD20) positive, CD10 positive, B-cell lymphoma (Bcl)-2 positive, Bcl-6 positive, CD5 negative, CD3 negative, melanoma marker (S100) negative, cytokeratin-7 negative, and exhibited a high mitotic index marker (Ki-67= 80%). According to the immunohistochemical (IHC) findings, we concluded that this patient has a clear cell variant of diffuse large B-cell lymphoma of germinal cell type. Our patient is undergoing R-CHOP chemotherapy.

Keywords: Cancer; B-cell lymphoma; Cell morphology; Cytokeratins.

1. Introduction

Diffuse large B-cell lymphoma (DLBCL) is an aggressive, rapidly growing neoplasm composed of lymphoid cells with a nucleus comparable in size to or larger than that of a reactive histiocyte [1]. DLBCL displays prominent heterogeneity at the clinical, histological and molecular levels [2]. DLBCL is the most common type of non-Hodgkin lymphoma affecting people worldwide, accounting for nearly 5 new cases per 100,000 persons [3].

Large B-cell lymphoma with clear cells is a rare morphological variant of the diffuse large B-cell lymphoma (DLBCL) according to classification system of Revised European American Lymphoma (REAL) and the classification established by World Health Organization (WHO) in 2008 [3,4]. The aim of different classifications were to grouping together all malignant lymphomas characterized by the large size of the neoplastic cells of B-cell origin as well as by an aggressive clinical presentation [5,6]. Immunophenotype, tissue microarray and molecular studies highlight the prominent heterogeneity of DLBCLs and suggest a sub-classification of the tumor based on the identification of different pathogenic pathways; this might have much greater significance than pure morphology for accurate prognostic expectation [6].

2. Case presentation

A 50-year-old Libyan woman from Misurata presented with rapid growing and painless enlargement of cervical lymph node, but she also disclosed fatigue and B symptoms including fever, night sweats and weight loss. Our patient underwent a cervical lymph node biopsy (core biopsy) at Misurata Cancer Center. According to the first biopsy findings, based on hematoxylin and eosin-stained slides created by co-author (DA), the diagnosis was suspected of being a cervical lymph node metastatic carcinoma; therefore, it was investigated for primary carcinoma, which was not identified. Clinically and radiographically, the mediastinum and kidneys were clear. This case has been reviewed by the authors (WAE and FBA), and by using appropriate immunohistochemical studies, arrived at the final diagnosis of a clear cell variant of DLBCL.

3. Results and Discussion

The lymph node biopsy showed a partially alveolar growth pattern and marked sclerosis (Figure 1), which raised clinical suspicions of an epithelial neoplasm. The morphological and phenotypic features comprised large nodules in diffuse areas, composed of large cells with slightly irregular nuclei and clear cytoplasm admixed with a few mononuclear cells, as well as sheets of large cells with abundant pale cytoplasm separated by collagenous fibrosis. The nuclei were round (centroblast-like) or sometimes multi-lobulated (Figure 1). These areas displayed high mitotic activity.

Staining for cytokeratins (CK) was negative. These areas disclosed the following phenotype: cluster designation marker 20 (CD20) expressed strong positivity (Figure 2.A), CD10 positive (Figure 2.B), B-cell

lymphoma (Bcl-2) expressed cytoplasmic staining, CD5-negative, CD3-negative, melanoma marker S100-negative (Figure 3.A), and Ki-67 expressed a high proliferation index of 70%. (Figure 3.B). Based on the histological examination, the differential diagnosis of the tumor needed to include other lympho-proliferative conditions in which large B-cells could be observed. Our first thought was the possibility of primary mediastinal large B-cell lymphoma (PMBCL), but clinically no tumor mass was found in the mediastinum. Radiography of the mediastinum did not show any pathological change. The IHC results excluded clinical suspicions of a metastatic tumor.

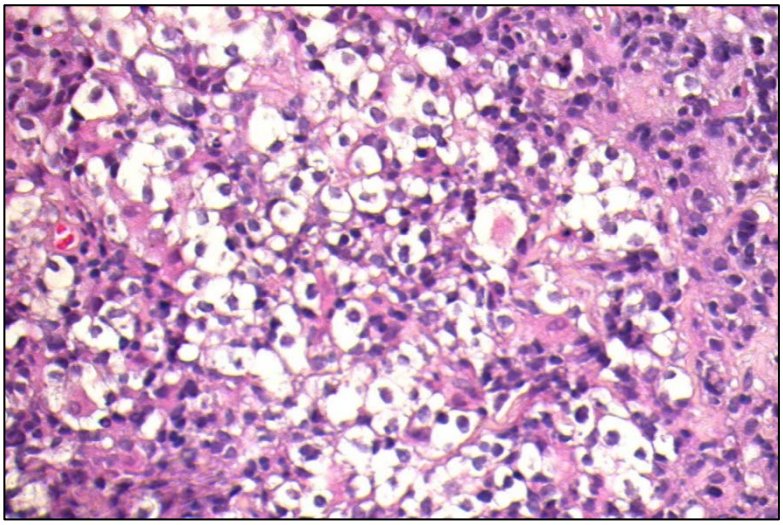

Figure (1): The neoplastic cells exhibited clear cell morphology with some cells revealed granular eosinophilic cytoplasm and embedded within vascular stroma (H and E×400)

The differential diagnosis following the first round of IHC included uncommon, undifferentiated large-cell carcinoma, malignant melanoma and undifferentiated mesenchymal large-cell neoplasms. In the second round of IHC, we considered the possibility of DLBCL (The morphological variants are centroblastic, immunoblastic, T-cell- and histiocyte-rich, anaplastic, plasmablastic, DLBCL-anaplastic lymphoma kinase-positive and PMBCL). Our final diagnosis was of a clear cell variant of DLBCL.

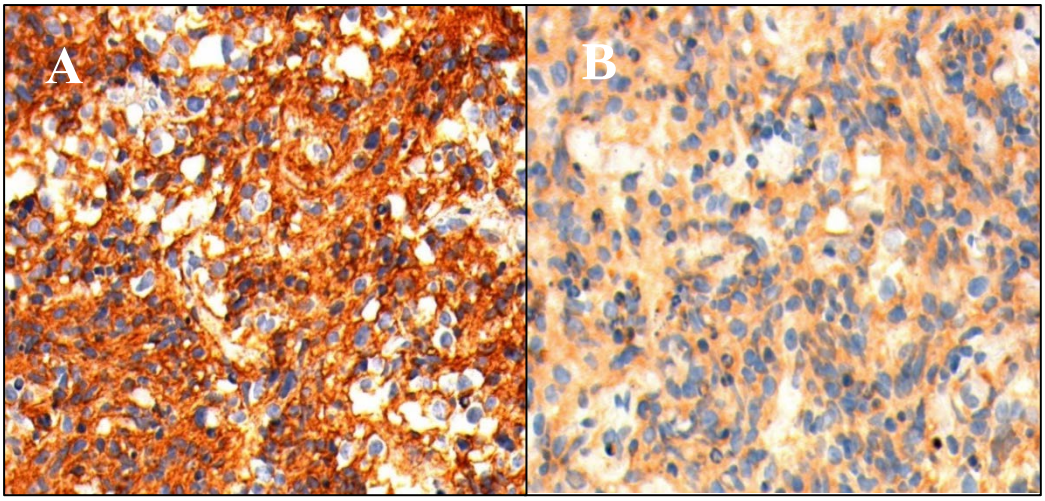

Figure (2): DLBC Lymphoma shows neoplastic cells stained intensely (A) for membranous CD20 and (B) stained moderately for membranous CD10 (400XDAB).

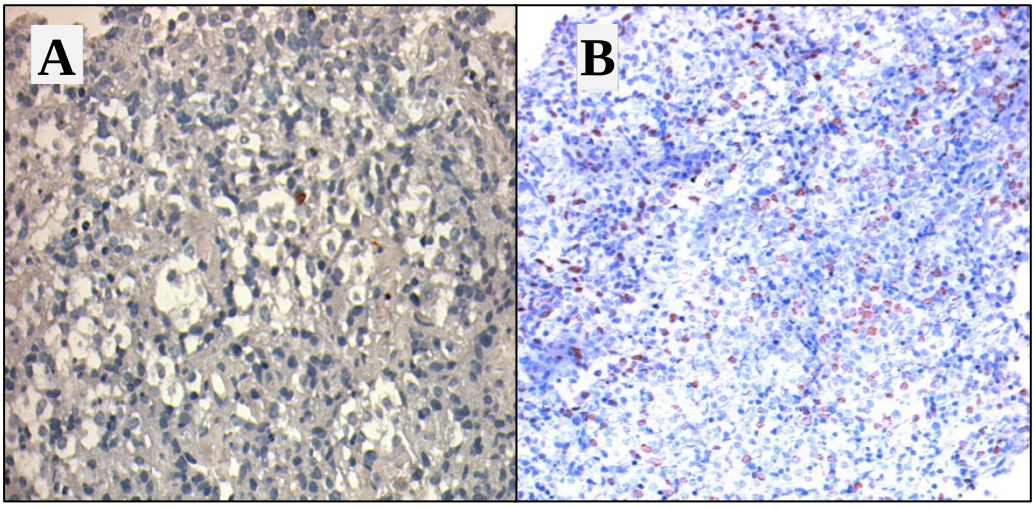

Figure (3): DLBC Lymphoma shows neoplastic cells negatively stained for cytoplasmic S 100 (A). Whereas (B) about 70% nuclear-staining by the MIB1; IgG monoclonal antibody used for detection Ki-67 in paraffin embedded material, which indicates high mitotic index (200XDAB).

Diffuse Large B - Cell Lymphoma (DLBCL) is a heterogeneous category of Mature B- Cell Neoplasms in the updated 2008 WHO Classification[4]. The current case had to be differentiated from T-cell histiocyte-rich large B-cell lymphoma (TCHRLBCL), which shows CD20+,

CD30-, CD15-, almost no small CD20+ or immunoglobulin D-positive (IgD+) B-cells. Furthermore, it frequently shows more CD8+ than CD4+ T-cells in the background [6]. On other hand, using various immunohistochemical antibodies, such as CD10, CD138, anti-Bcl-2, anti-Bcl-6, MUM1 and anti-p53, several groups have tried to sub-classify DLBCL into the germinal center B-cell-like DLBCL (GCB-DLBCL) and activated B-cell-like DLBCL (ABC-DLBCL) sub-groups, with comparable differences in clinical behavior. Alizadeh et al. recognized two molecularly distinct types of DLBCL that had gene expression patterns indicative of different stages of B cell differentiation [7]. Some patients expressed genes pattern A (GCB-DLCBL), and others expressed genes pattern B (ABC-DLBCL). Patients with GCB-DLCBL had better outcome than those with non-GCB subtype [8]. On other hand, both A and B gene patterns show a significant improvement of overall survival rate after rituximab, cyclophosphamide, doxorubicin, Oncovin (vincristine sulfate) and prednisolone (RCHOP) chemotherapy [9]. Our patient with GCB-DLBCL undergoing R-CHOP treatment. Over-expression of Bcl-6, Bcl-2 and cMyc proteins, which phenotypes of the presence Bcl-6 Bcl-6, Bcl-2 and cMyc genotypes abnormality, which may have significant roles in the pathogenesis and progression of DLBCL subclass [7, 10]. Patients with pattern B (ABC-DLBCL) revealed higher mitotic index than those with pattern A (GCB-DLBCL). These findings suggest that high proliferation activity of that lymphoma with pattern B may be associated with aggressive tumor behavior and poor prognosis [8]. Additional novel therapies under investigation include those monoclonal antibody-based therapy which targeting both Bcl-6 and Bcl-2 is underway [11].

4. Conclusion

It has become clear that diffuse large B-cell lymphoma is not a uniform category. Several recognizable variants have clinically distinct features and, frequently, require specific treatment approaches. Recognition of these variants and utilization of the appropriate treatments will improve the outcome for the patients. Based on IHC findings, it was concluded that the diagnosis in the present case is a clear cell variant of DLBCL of germinal cell type. Our patient is alive and undergoing R-CHOP chemotherapy.

Ethical approval

All authors hereby declare that the proposed study has examined and approved by the Research Council of Misurata Cancer Center.

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The Association of Childhood Asthma and Breastfeeding and Other Independent Risk Factory

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Abstract

The aim of the study is to investigate the association of breastfeeding duration and incidence of childhood asthma in misurata central hospital. To assess the effect of positive family history (first degree), L.B.W, indoor smoking, socioeconomic states (maternal education, family income) and the race in developing bronchial asthma. This study is carried out in pediatric department and follow up clinics at misurata central hospital. Study population was children who came to the hospital with different complain. Study carried out during period of 6 months and special questionnaire was done as mentioned later on. Study population divided to the 2 main groups; first group (children who have asthma) that studied with different risk factor second group (children who have no asthma). Among the studied groups, the incidence of childhood asthma is as a high as 23% of non breastfeed babies compared to as a low as 7% of babies who were breastfeed for one year or longer. Effect of other Risk factors on incidence of asthma were as follows: 1) 32% of the asthma cases were attribute to the low birth weight; 2) children with environmental tobacco smoke (ETS) exposure (39%) had higher incidence of asthma than did those from smoke free homes; 3) there is no consistent correlation between family income and incidence of asthma, asthma was equal in both girls & boys, the incidence was higher among Hispanic children than whites & blacks and it was highest in the age group between 1-5 years; 4) 27% of patients had positive family history and 36% of patients had other atopic disorders. The results of this study shows that breast feeding and in particular longer duration is protective against childhood asthma, So New mothers should be encouraged to breast feed as long as possible, since never breast feeding, breastfeeding for less them 3 moths (1-90 days) may be an independent risk factor for childhood asthma. Low birth weight children and exposure to environmental tobacco smoke (ETS) are considered to be important independent risk factors for development of later childhood asthma, so restrictive measures should be taken to avoid any factor which leads to LBW infants and any steps to stop smoke. There is no consistent relationship between childhood asthma and socioeconomic state.

Keywords: Childhood asthma; Breastfeeding duration; LBW; Socioeconomic state; Risk factors; Family history of asthma.

1. Introduction

Asthma is the most common chronic disorder of childhood throughout the world. The prevalence of asthma varies from 5 – 20%. 30 % of children

also have a form of asthma at some age. ISAAC found that the United Kingdom, Australia, New Zealand had among the highest prevalence's with 15 % children affected. Boys 14% vs girls 10%, poor families 16% vs not poor families 10% are more likely to have asthma. The most common patterns of childhood wheezing illnesses are: episodic viral wheeze 60%; asthma 40% (atopic asthma 85% and non atopic asthma 15%); third type childhood asthma emerges in females who develop obesity and early onset of puberty (by 11 years of age). Breastfeeding is good and healthy for infants and moms. It has disease fighting cells and antibodies that help to protect infants from germs, illness and even SIDS [1].

Breastfeeding lowers risk of following health problem:

1) In infants
   Respiratory and ear infection - GIT viruses (AGE), diarrhea.
   Asthma and a topic dermatitis - Obesity and type 1 and 2 DM.
   Childhood leukemia and SIDS - Necrotizing enterocolitis.
   Enhancement of cognitive development.
2) In Moms
   Type (Dm_2)
   Breast cancer and ovarian cancer
   Postpartum depression.

Breastfeeding is widely advocated to reduce risk of asthma and atopy but the evidence for protective effect of breastfeeding on asthma and other allergic disease in childhood is inconclusive, some studies have reported greater degrees of protection with exclusive and prolonged breastfeeding [2-5] and several studies have noted a larger protection effect in children prone to atopy [6,7], other studies however have reported no reduction in risk or even an increase in risk with breastfeeding [8-14]. Australia has one of the highest asthma rate in the world, some factors that maight have contributed to the rise in childhood asthma over the past decade include: less women breastfeeding especially after 3 months of age, increase exposure to the viral infection while young especially early in life (changes in the life style, not enough oil-fish in Australian diet). The risk factors that contribute to both the expression and persistence of asthma include: 1) family history of atopy and co-existence of atopic disease; 2) birth weight and prematurity; 3) parental smoking; 4) breastfeeding; 5) effect of sex; 6) bronchiolitis in infancy; 7) younger age at presentation.

2. Materials and Methods

A Descriptive Retrospective study was carried out in pediatric department and fallow up clinics at misurata central hospital. Study population who underwent investigation and assessment were (2518) children aged between (6 months – 14 years) came to the department to seek medical help from different complains. This study carried out during period (1. 9. 2009 to the 28. 2. 2010). Many doctors shared in this study by filling out the data which present in special questionnaire prepared especially for this study. The questionnaire include the following data as: duration of breastfeeding, asthma yes/no, positive family history of asthma, LBW, indoor parent smoking, socioeconomic state (income, maternal education), race, sex. These large number of children divided to the two main groups, one group, children who had asthma and second group, children who had no asthma with prolonged intervals of breastfeeding (> 6 months and > 12 months).

3. Results and Discussion

Out of (2518) children, (209) children had asthma, incidence 8.3%. Duration of breastfeeding to the incidence of asthma shows inverse association with increase duration of breastfeeding the incidence of asthma decrease, for example, breast feeding up to 90 days, asthmatic cases were 56 cases out of 209 cases (27%) in comparison to the children who had breastfeeding up to 356 days. The asthmatic cases were 20 out of 209 (9.5%) Table (1). Highest incidence of asthma were in children aged from 6 months to the 5 years were 126 out of 209 cases (60%) and lowest incidence were in children between 9 – 14 years, about 38 cases (18.2%) Table (2) Incidence of asthma was highest among Hispanic children, 103 cases out of 209 cases (49.3%) and among white children was 74 cases, (35.4%) and lowest among blackest, 32 cases (15.3%) Table (3). 56 cases (27%) had positive family history of asthma either first or second degree and 153 cases (73%) had negative family history Table (4).

Birth weight less than 2.5 kg (LBW) was associated with increased risk of asthma 67 cases out of 209 cases were BW less than 2.5 kg (32%) Table (5). In this study, number of asthmatic children who had history of exposure to the tobacco smoke (ETS) were 82 cases (39%) Table (6). Table 7 showed no consistent association between family income and the incidence of asthma, in low income family number of asthmatic children were 37 cases (35%) and in families with moderate income number of children who had asthma were 104 cases (49%) and with good income families percent of asthmatic children were (32 cases) 16%. Most common symptoms present in these asthmatic children was shortness of breathing

(151 cases) 72%, and less common symptom was cough (70 cases) 33%, worsening of symptoms at night present in (148 cases) (68%) Table (9). Association of asthma with other atopic disorders were positive in 74 cases (36%), as:

- Allergic rhinitis (32 cases) 15.5 %.
- Eczema of skin (28 cases) 13.5 %.
- Allergic conjunctivitis (14 cases) 7%.

The incidence of asthma were higher in boys, 118 cases (56.5%) than girls 91 cases (43.5%) Table (2).

Table (1)

Breastfeeding	Case of asthma	%
Nil	48	23%
1 – 90 days	56	27%
91 – 180 days	41	20 %
181– 270 days	28	13.5%
271 – 356 days	20	9.5 %
256 days	16	7%

Table (2)

Age	Male	%	Female	%	Total	%
6 mo-2y	38	18	35	16.5	73	34.5
3 – 5y	29	14	24	11.5	53	25.5
6 – 8 y	28	13.5	17	7.5	45	21
9 – 11 y	16	7.5	9	4.5	25	12
12 – 14 y	7	4	6	3	13	7

Table (3)

Race	Male	%	Female	%	Total	%
White	46	22	28	13	74	35.4
Black	14	1.6.5	18	8.5	37	15.3
Hispanic	46	22	57	28	103	49

Table (4)

Family History	Total number of asthma	%
- Ve family history	153	37
+ Ve family history	56	27
First degree	34	16.5
Second degree	22	10.5

Table (5)

Birth weight	Male	%	Female	%	Total	%
> 2.5 kg	21	10	46	22	67	32
2.5 -3.5 kg	64	31	39	18	103	49
< 3.5 kg	18	9	21	10	39	19

Table (6)

Environmental tobacco smoke (ETS)	Number case of asthma	%
Parent smoke + Ve	82	39
Parent smoke - Ve	127	61

Table (7)

Family income	Number case of asthma	%
> 500 D.L Low income	73	35
500 – 1000 D.L Moderate income	104	49
< 1000 good income	32	16

Table (8)

Symptoms	Percent	Present	Not present
Coughing	33%	70	139
Wheezing	41%	86	123
Short breathing	72%	151	58
Worsening of symptoms at Night	68 %	148	61

Table (9)

Allergic disease	No	%
- Ve Allergic disease	135	64
+ Ve Allergic disease	74	36
Allergic Rhinitis	32	15.5
Eczema of skin	28	13.5
Allergic conjunction	14	7

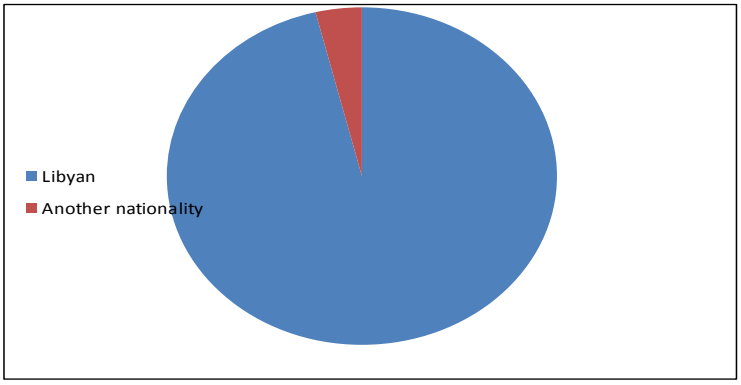

Figure (1): Number of Libyan and other nationalities.

Present study mainly investigated the relationship of breastfeed duration and the incidence of childhood asthma, the results of this study support the researches that show breastfeeding is protective against childhood asthma. Our study shows significant reduction in incidence of childhood asthma with prolonged breastfeeding, infants who were breastfeed for < 90 days had high risk of asthma compared to those who had been breastfeed for a year or longer (who had low risk).

Several studies have reported that a significant reduction of childhood asthma has been associated for at least 4–6 months of exclusive breastfeeding [15,16], many other investigators claimed that breastfeeding is highly protective against asthma [17,18]. Few reports shown that, the effect of breastfeeding was more evident in boys than girls [19], and was seen only among children with no family history of asthma [20]. We also found that environmental tobacco smoking (ETS) exposure was associated with high incidence of childhood asthma. In our study, 39% of children who had asthma had history of ETS exposure, this finding is in accordance with previously reported study [21]. The study reported that child with ETS exposure (37.9%) had higher incidence of asthma than did those from smoke free homes.

There is also strong evidence that exposure to ETS in childhood causes chronic respiratory symptom as cough and wheeze and a cute and chronic otitis media and it has a causal role in childhood asthma [22]. After considering factors that contribute to development of asthma, the strongest independent risk factor for asthma was low birth weight (<2.5kg), recent study (2006) published in the American Journal of public health reported that LBW children are at high risk to develop asthma (36%) [23]. In our study, 32% of asthmatic children were LBW compared to study conducted previously [24]. Statistical analysis suggested that 31% of asthmatic cases were attributable to low birth weight.

There is conflicting information about the relationship between the asthma and socioeconomic state, different studies reported no significant impact on the incidence of asthma [25]. In our study there is no relationship between development of asthma and family income or maternal education (no consistent association). In early life the incidence of asthma is higher in boys, at puberty however the sex ratio shifts and asthma appears predominantly in females. In present study, the incidence of asthma in boys

was 56.5% and in girls was 43.5% (up to 14 years of age) correlates well with previous studies.

4. Conclusion

The results of our study shows that breastfeeding and in particular longer duration is protective against childhood asthma. Low birth weight infants and exposure to the environmental tobacco smoke (ETS) are considered to be important independent risk factors for development of childhood asthma. There is no consistent association between socioeconomic stat and childhood asthma. The incident of childhood asthma is higher among boys than girls, higher among children aged 1 – 5 years than others and higher among Hispanic than whites and blacks. Breastfeed more than 4 months is highly recommended since never breastfeeding or breastfeeding less than 3 months, (90 days) may be an independent risk factor for childhood asthma.

Recommendation

New mothers should be encouraged to breastfeed for as long as possible (minimum more than 4 – 6 months), long duration breastfeeding reduces child risk to develop asthma, more public health efforts should be directed toward increasing the initiation and duration of breastfeeding, if exclusive breastfeeding is not possible, supplementation with cow's milk formula is recommended .Parents should be advised not to smoke and to avoid children being exposed to other people's smoke. All risk factors which attributed to low birth weight and prematurity should be avoided if possible by improve the antenatal care for pregnant women.

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